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(71) Applicant *(for all designated States except US)*: **EXPRESSION DIAGNOSTICS, INC.** [US/US]; 384 Oyster Point Boulevard, Suite No. 6, South San Francisco, CA 94080 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **WOHLGEMUTH, Jay** [US/US]; 1165 Monte Rosa Drive, Menlo Park, CA 94025 (US). **FRY, Kirk** [US/US]; 2604 Ross Road, Palo Alto, CA 94303 (US). **WOODWARD, Robert** [US/US]; 1828 Rheem Court, Pleasanton, CA 94588 (US). **LY, Ngoc** [US/US]; 2000 Crystal Springs Road 15-14, San Bruno, CA 94066 (US).

(74) Agents: **LITTLEFIELD, Otis, B.** et al.; Morrison & Foerster LLP, 425 Market Street, San Francisco, CA 94105-2482 (US).

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(54) **Title:** METHODS AND COMPOSITIONS FOR DIAGNOSING AND MONITORING AUTO IMMUNE AND CHRONIC INFLAMMATORY DISEASES

(57) **Abstract:** Methods of diagnosing or monitoring auto immune and chronic inflammatory diseases, particularly systemic lupus erythematosis and rheumatoid arthritis, in a patient by detecting the expression level of one or more genes in a patient, are described. Diagnostic oligonucleotides for diagnosing or monitoring auto immune and chronic inflammatory diseases, particularly systemic lupus erythematosis and rheumatoid arthritis and kits or systems containing the same are also described.

METHODS AND COMPOSITIONS FOR DIAGNOSING AND MONITORING AUTO IMMUNE AND CHRONIC INFLAMMATORY DISEASES

Related Applications

This application claims priority to U.S. Patent Application number 10/131,827 filed April 24, 2002, which is hereby incorporated by reference in its entirety.

Field of the Invention

This application is in the field of chronic inflammatory diseases. In particular, this invention relates to methods and compositions for diagnosing or monitoring chronic inflammatory diseases.

Background of the Invention

Many of the current shortcomings in diagnosis, prognosis, risk stratification and treatment of disease can be approached through the identification of the molecular mechanisms underlying a disease and through the discovery of nucleotide sequences (or sets of nucleotide sequences) whose expression patterns predict the occurrence or progression of disease states, or predict a patient's response to a particular therapeutic intervention. In particular, identification of nucleotide sequences and sets of nucleotide sequences with such predictive value from cells and tissues that are readily accessible would be extremely valuable. For example, peripheral blood is attainable from all patients and can easily be obtained at multiple time points at low cost. This is a desirable contrast to most other cell and tissue types, which are less readily accessible, or accessible only through invasive and aversive procedures. In addition, the various cell types present in circulating blood are ideal for expression profiling experiments as the many cell types in the blood specimen can be easily separated if desired prior to analysis of gene expression. While blood provides a very attractive substrate for the study of diseases using expression profiling techniques, and for the development of diagnostic technologies and the identification of therapeutic targets, the value of expression profiling in blood samples rests on the degree to which changes in gene expression in these cell types are associated with a predisposition to, and pathogenesis and progression of a disease.

There is an extensive literature supporting the role of leukocytes, e.g., T-and B-lymphocytes, monocytes and granulocytes, including neutrophils, in a wide range of disease processes, including such broad classes as cardiovascular diseases, inflammatory, autoimmune and rheumatic diseases, infectious diseases, transplant rejection, cancer and malignancy, and endocrine diseases.

Of particular interest is the role of leukocytes and leukocyte gene expression in chronic inflammatory diseases such as Systemic Lupus Erythematosis and Rheumatoid Arthritis. Systemic lupus erythematosis (SLE) and Rheumatoid Arthritis (RA) are chonic autoimmune and inflammatory disorders characterized by dysregulation of the immune system, which causes damage to a variety of organs. These diseases clearly involve differential expression of genes in leukocytes. Diagnostic and disease monitoring tools are severly lacking for these patients and their physicians. Leukocyte expression profiling can be applied to discover expression markers for SLE and RA and apply them as patient management tools in the clinical setting. In addition, osteoarthirtis is a degenerative joint

disease that can be confused with RA. This disease also involves leukocytes and expression profiling of leukocytes associated with osteoarthritis may lead to the discovery of new diagnostic and therapeutic approaches to the disease.

SLE in particular is a chronic, usually life-long, potentially fatal autoimmune disease characterized by unpredictable exacerbations and remissions with protean clinical manifestations. SLE is notable for unpredictable exacerbations and remissions and a predilection for clinical involvement of the joints, skin, kidney, brain, serosa, lung, heart, and gastrointestinal tract. The pathologic hallmark of the disease is recurrent, widespread, and diverse vascular lesions.

SLE is not a rare disorder. Although reported at both extremes of life (e.g. diagnosed in infants and in the tenth decade of life) chiefly it affects women of child bearing age. Among children, SLE occurs three times more commonly in females than in males. In the 60% of SLE patients who experience onset of their disease between puberty and the fourth decade of life the female to male ratio is 9:1. Thereafter, the female preponderance again falls to that observed in prepubescents.

The disorder is three times more common in African American blacks than American caucasians. SLE is also more common in Asians and in China may be more common than Rheumatoid Arthritis. The ethnic group at greatest risk is African Caribbean blacks. The annual incidence of SLE ranges from six to 35 new cases per 100,000 population in relatively low-risk to high-risk groups. The prevalence of SLE in the United States is an issue of some debate. Prevalence estimates of between 250,000 to 500,000 are contradicted by a recent nationwide telephone poll suggesting a prevalence of between one and two million.

The prognosis for patients with SLE has greatly improved over the last few decades with at least 80-90% of all patients surviving ten years. Thereafter life expectancy approximates that of age matched controls. This improvement reflects the general advancements in health care (i.e. dialysis, antibiotics, antihypertensives, newer immunosuppressives with more favorable efficacy to toxicity ration) but also the specialized care available for patients with SLE.

Such specialized medical care includes care by experienced clinicians with access to state of the art diagnostic and therapeutic measures will result in improved outcomes and the most cost-effective utilization of resources. Expert care of patients with SLE leads to fewer hospitalizations secondary to uncontrolled disease exacerbation, less severe renal disease with fewer patients experiencing end stage renal disease requiring chronic dialysis, fewer episodes of avascular necrosis requiring total joint replacement, and less severe osteoporosis and fractures. In addition, more judicious use of steroids and steroid sparing agents can also reduce the severity of atherosclerosis and resulting incidence of myocardial infarctions and cerebral vascular accidents, which now complicate the natural history of SLE. Improved monitoring, diagnosis and prognosis of SLE should aid clinicians in determining appropriate care for SLE patients, including which drugs to use and at what amounts.

At a molecular level, SLE is an autoimmune disease characterized by immune dysregulation resulting in the production of antinuclear antibodies (ANA), generation of circulating immune complexes, and activation of the complement system. SLE is futher characterized by end organ damage that results from deposition of circulating autoantibodies and subsequent complement- and Fc receptor-mediated inflammation. In addition, extensive immune system abnormalities, including altered

T lymphocyte function and spontaneous apoptosis, contribute to the lymphopenia and increased susceptibility to infection that confer considerable morbidity.

The clinical features of SLE are protean and may mimic infectious mononucleosis, lymphoma, or other systemic disease. Therefore, the American College of Rheumatology developed criteria to include patients with SLE and exclude those with other disorders. These criteria are best used to insure the appropriateness of subjects for epidemiological or research studies. Although many patients do not fulfill the rigid criteria at first encounter most will when followed over periods of time.

The etiology of SLE remains unknown. A genetic predisposition, sex hormones, and environmental trigger(s) likely result in the disordered immune response that typifies the disease.

A role for genetics is suggested by the increased percentage of two histocompatibility antigens in patients with SLE, HLA-DR2 and HLA-DR3. In addition, there is an increased frequency of the extended haplotype HLA-A1, B8, DR3. The role for heredity is further supported by the concordance for this illness among monozygotic twins. The polygenic nature, however, of this genetic predisposition as well as the contribution of environmental factors is suggested by the only moderate concordance rate which is reported to be between 25 and 60%.

The origin of autoantibody production in SLE is unclear but a role has been suggested for an antigen driven process, spontaneous B-cell hyper-responsiveness, or impaired immune regulation. Regardless of the etiology of autoantibody production, SLE is associated with the impaired clearance of circulating immune complexes secondary to decreased CR1 expression, defective Fc receptor function, or deficiencies of early complement components such as C4A.

More is known about the pathogenic cellular and molecular events that are responsible for vascular lesions in SLE than the origins of autoimmunity. Disease manifestations result from recurrent vascular injury due to immune complex deposition, leukothrombosis, or thrombosis. Additionally, cytotoxic antibodies can mediate autoimmune hemolytic anemia and thrombocytopenia, while antibodies to specific cellular antigens can disrupt cellular function. An example of the latter, is the association between anti-neuronal antibodies and neuropsychiatric SLE.

The health status of a patient with SLE is related not only to disease activity, but to the damage that results from recurrent episodes of disease flare (i.e. deforming arthropathy, shrinking lung, end stage renal disease, organic mental syndrome, etc.), as well as the adverse effects of treatment (i.e. avascular necrosis of bone, infections, and precocious atherosclerosis, etc.).

The accuracy of technologies based on expression profiling for the diagnosis, prognosis, and monitoring of disease would be dramatically increased if numerous differentially expressed nucleotide sequences, each with a measure of sensitivity and specificity for a disease in question, could be identified and assayed in a concerted manner. Using the expression of multiple genes (gene sets) for diagnostic applications helps overcome assay and population variability. PCT application WO 02/057414 "LEUKOCYTE EXPRESSION PROFILING" to Wohlgemuth identifies a set of differentially expressed nucleotides.

In order to achieve this improved accuracy, the appropriate sets of nucleotide sequences once identified need to be validated against numerous samples in combination with relevant clinical data.

Summary of the Invention

In order to meet these needs, the present invention identifies genes and gene sets that have clinical utility as diagnostic tools for the management of lupus patients and patients with a variety of chronic inflammatory and autoimmune diseases. The present invention is thus directed to a method of diagnosing or monitoring chronic autoimmune or inflammatory disease in a patient. The method of the invention involves detecting in a patient expression of one or more genes such as those genes depicted in Table 2E and surrogates derived therefrom. Exemplary surrogates are provided in Table 2D. The present invention is further directed to a method of diagnosing or monitoring an autoimmune or chronic inflammatory disease in a patient by detecting the expression level of one or more genes or surrogates derived therefrom in said patient to diagnose or monitor the autoimmune or chronic inflammatory disease in the patient wherein said genes include a nucleotide sequence selected from SEQ ID NO:503, SEQ ID NO:504, SEQ ID NO:505, SEQ ID NO:506, SEQ ID NO:507, SEQ ID NO:508, SEQ ID NO:509, SEQ ID NO:510, SEQ ID NO:511, SEQ ID NO:512, SEQ ID NO:513, SEQ ID NO:514, SEQ ID NO:515, SEQ ID NO:516, SEQ ID NO:517, SEQ ID NO:518, SEQ ID NO:519, SEQ ID NO:520, SEQ ID NO:521, SEQ ID NO:522, SEQ ID NO:523, SEQ ID NO:524, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:540, SEQ ID NO:541, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, SEQ ID NO:545, SEQ ID NO:546, SEQ ID NO:547, SEQ ID NO:548, SEQ ID NO:549, SEQ ID NO:550, SEQ ID NO:551, SEQ ID NO:552, SEQ ID NO:553, SEQ ID NO:554, SEQ ID NO:555, SEQ ID NO:556, SEQ ID NO:557, SEQ ID NO:558, SEQ ID NO:559, SEQ ID NO:560, SEQ ID NO:561, SEQ ID NO:562, SEQ ID NO:563, SEQ ID NO:564, SEQ ID NO:565, SEQ ID NO:566, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:569, SEQ ID NO:570, SEQ ID NO:571, SEQ ID NO:572, SEQ ID NO:573, SEQ ID NO:574, SEQ ID NO:575, SEQ ID NO:576, SEQ ID NO:577, SEQ ID NO:578, SEQ ID NO:579, SEQ ID NO:580, SEQ ID NO:581, SEQ ID NO:582, SEQ ID NO:583, SEQ ID NO:584, SEQ ID NO:585, SEQ ID NO:586, SEQ ID NO:587, SEQ ID NO:588, SEQ ID NO:589, SEQ ID NO:590, SEQ ID NO:591, SEQ ID NO:592, SEQ ID NO:593, SEQ ID NO:594, SEQ ID NO:595, SEQ ID NO:596, SEQ ID NO:597, SEQ ID NO:598, SEQ ID NO:599, SEQ ID NO:600, SEQ ID NO:601, SEQ ID NO:602, SEQ ID NO:603, SEQ ID NO:604, SEQ ID NO:605, SEQ ID NO:606, SEQ ID NO:607, SEQ ID NO:608, SEQ ID NO:609, SEQ ID NO:610, SEQ ID NO:611, SEQ ID NO:612, SEQ ID NO:613, SEQ ID NO:614, SEQ ID NO:615, SEQ ID NO:616, SEQ ID NO:617, SEQ ID NO:618, SEQ ID NO:619, SEQ ID NO:620, SEQ ID NO:621, SEQ ID NO:622, SEQ ID NO:623, SEQ ID NO:624, SEQ ID NO:625, SEQ ID NO:626, SEQ ID NO:627, SEQ ID NO:628, SEQ ID NO:629, SEQ ID NO:630, SEQ ID NO:631, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:634, SEQ ID NO:635, SEQ ID NO:636, SEQ ID NO:637, SEQ ID NO:638, SEQ ID NO:639, SEQ ID NO:640, SEQ ID NO:641, SEQ ID NO:642, SEQ ID NO:643, SEQ ID NO:644, SEQ ID NO:645, SEQ ID NO:646, SEQ ID NO:647, SEQ ID NO:648, SEQ ID NO:649, SEQ ID NO:650, SEQ ID NO:651, SEQ ID NO:652, SEQ ID NO:653, SEQ ID NO:654, SEQ ID NO:655, SEQ ID NO:656, SEQ ID NO:657, SEQ ID NO:658, SEQ ID NO:659, SEQ ID NO:660, SEQ ID NO:661, SEQ ID NO:662, SEQ ID NO:663, SEQ ID NO:664, SEQ ID NO:665, SEQ ID NO:666, SEQ ID NO:667, SEQ

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In the method of the invention, the diagnosing of monitoring may be performed by detecting the expression level of two or more genes, three or more genes, four or more genes, five or more genes, six or more genes, seven or more genes, eight or more genes, nine or more genes, ten or more genes, fifteen or more genes, twenty or more genes, thirty or more genes, fifty or more genes, one hundred or more genes, two hundred or more genes, or all five hundred and two of the genes.

The methods of the invention also includes diagnosing or monitoring auto immune and chronic inflammatory diseases in a patient by detecting the expression level of one or more genes in said patient to diagnose or monitor auto immune and chronic inflammatory diseases in said patient wherein said one or more genes identified by a nucleotide sequence selected from the following group: SEQ ID NO:503, SEQ ID NO:505, SEQ ID NO:506, SEQ ID NO:508, SEQ ID NO:509, SEQ ID NO:510, SEQ ID NO:511, SEQ ID NO:512, SEQ ID NO:513, SEQ ID NO:514, SEQ ID NO:515, SEQ ID NO:516, SEQ ID NO:517, SEQ ID NO:518, SEQ ID NO:519, SEQ ID NO:520, SEQ ID NO:521, SEQ ID NO:522, SEQ ID NO:523, SEQ ID NO:524, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:540, SEQ ID NO:541, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, SEQ ID NO:545, SEQ ID NO:546, SEQ ID NO:547, SEQ ID NO:548, SEQ ID NO:549, SEQ ID NO:550, SEQ ID NO:551, SEQ ID NO:552, SEQ ID NO:553, SEQ ID NO:554, SEQ ID NO:555, SEQ ID NO:556, SEQ ID NO:557, SEQ ID NO:558, SEQ ID NO:559, SEQ ID NO:560, SEQ ID NO:561, SEQ ID NO:562, SEQ ID NO:563, SEQ ID NO:564, SEQ ID NO:565, SEQ ID NO:566, SEQ

ID NO:567, SEQ ID NO:568, SEQ ID NO:569, SEQ ID NO:570, SEQ ID NO:571, SEQ ID NO:572, SEQ ID NO:573, SEQ ID NO:574, SEQ ID NO:575, SEQ ID NO:576, SEQ ID NO:577, SEQ ID NO:578, SEQ ID NO:579, SEQ ID NO:580, SEQ ID NO:581, SEQ ID NO:582, SEQ ID NO:583, SEQ ID NO:584, SEQ ID NO:585, SEQ ID NO:586, SEQ ID NO:587, SEQ ID NO:588, SEQ ID NO:589, SEQ ID NO:590, SEQ ID NO:591, SEQ ID NO:592, SEQ ID NO:593, SEQ ID NO:594, SEQ ID NO:595, SEQ ID NO:596, SEQ ID NO:597, SEQ ID NO:598, SEQ ID NO:599, SEQ ID NO:600, SEQ ID NO:601, SEQ ID NO:602, SEQ ID NO:604, SEQ ID NO:605, SEQ ID NO:606, SEQ ID NO:607, SEQ ID NO:608, SEQ ID NO:609, SEQ ID NO:610, SEQ ID NO:611, SEQ ID NO:612, SEQ ID NO:613, SEQ ID NO:614, SEQ ID NO:615, SEQ ID NO:616, SEQ ID NO:617, SEQ ID NO:618, SEQ ID NO:619, SEQ ID NO:620, SEQ ID NO:621, SEQ ID NO:622, SEQ ID NO:623, SEQ ID NO:624, SEQ ID NO:625, SEQ ID NO:626, SEQ ID NO:627, SEQ ID NO:628, SEQ ID NO:629, SEQ ID NO:630, SEQ ID NO:631, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:634, SEQ ID NO:635, SEQ ID NO:636, SEQ ID NO:637, SEQ ID NO:638, SEQ ID NO:639, SEQ ID NO:640, SEQ ID NO:641, SEQ ID NO:642, SEQ ID NO:643, SEQ ID NO:644, SEQ ID NO:645, SEQ ID NO:646, SEQ ID NO:647, SEQ ID NO:648, SEQ ID NO:649, SEQ ID NO:650, SEQ ID NO:651, SEQ ID NO:652, SEQ ID NO:653, SEQ ID NO:654, SEQ ID NO:655, SEQ ID NO:656, SEQ ID NO:657, SEQ ID NO:658, SEQ ID NO:659, SEQ ID NO:660, SEQ ID NO:661, SEQ ID NO:662, SEQ ID NO:663, SEQ ID NO:664, SEQ ID NO:665, SEQ ID NO:666, SEQ ID NO:667, SEQ ID NO:668, SEQ ID NO:669, SEQ ID NO:670, SEQ ID NO:671, SEQ ID NO:672, SEQ ID NO:673, SEQ ID NO:674, SEQ ID NO:675, SEQ ID NO:676, SEQ ID NO:677, SEQ ID NO:678, SEQ ID NO:679, SEQ ID NO:680, SEQ ID NO:681, SEQ ID NO:682, SEQ ID NO:683, SEQ ID NO:684, SEQ ID NO:685, SEQ ID NO:686, SEQ ID NO:687, SEQ ID NO:688, SEQ ID NO:689, SEQ ID NO:690, SEQ ID NO:691, SEQ ID NO:692, SEQ ID NO:693, SEQ ID NO:694, SEQ ID NO:695, SEQ ID NO:696, SEQ ID NO:697, SEQ ID NO:698, SEQ ID NO:699, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:705, SEQ ID NO:706, SEQ ID NO:707, SEQ ID NO:708, SEQ ID NO:709, SEQ ID NO:710, SEQ ID NO:711, SEQ ID NO:712, SEQ ID NO:713, SEQ ID NO:714, SEQ ID NO:715, SEQ ID NO:716, SEQ ID NO:717, SEQ ID NO:718, SEQ ID NO:719, SEQ ID NO:720, SEQ ID NO:721, SEQ ID NO:722, SEQ ID NO:723, SEQ ID NO:724, SEQ ID NO:725, SEQ ID NO:726, SEQ ID NO:727, SEQ ID NO:728, SEQ ID NO:729, SEQ ID NO:730, SEQ ID NO:731, SEQ ID NO:732, SEQ ID NO:733, SEQ ID NO:734, SEQ ID NO:735, SEQ ID NO:736, SEQ ID NO:737, SEQ ID NO:738, SEQ ID NO:739, SEQ ID NO:741, SEQ ID NO:742, SEQ ID NO:743, SEQ ID NO:744, SEQ ID NO:745, SEQ ID NO:746, SEQ ID NO:747, SEQ ID NO:748, SEQ ID NO:749, SEQ ID NO:750, SEQ ID NO:751, SEQ ID NO:752, SEQ ID NO:753, SEQ ID NO:754, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID NO:759, SEQ ID NO:760, SEQ ID NO:761, SEQ ID NO:762, SEQ ID NO:763, SEQ ID NO:764, SEQ ID NO:765, SEQ ID NO:766, SEQ ID NO:767, SEQ ID NO:768, SEQ ID NO:769, SEQ ID NO:770, SEQ ID NO:771, SEQ ID NO:772, SEQ ID NO:773, SEQ ID NO:774, SEQ ID NO:775, SEQ ID NO:776, SEQ ID NO:777, SEQ ID NO:778, SEQ ID NO:779, SEQ ID NO:780, SEQ ID NO:781, SEQ ID NO:782, SEQ ID NO:783, SEQ ID NO:784, SEQ ID NO:785, SEQ ID NO:786, SEQ ID NO:787, SEQ ID NO:788, SEQ ID NO:789, SEQ ID NO:790, SEQ ID NO:791, SEQ ID NO:792, SEQ ID NO:793, SEQ ID NO:794, SEQ ID NO:795,

SEQ ID NO:796, SEQ ID NO:797, SEQ ID NO:798, SEQ ID NO:799, SEQ ID NO:800, SEQ ID NO:801, SEQ ID NO:802, SEQ ID NO:803, SEQ ID NO:804, SEQ ID NO:805, SEQ ID NO:806, SEQ ID NO:807, SEQ ID NO:808, SEQ ID NO:809, SEQ ID NO:810, SEQ ID NO:813, SEQ ID NO:814, SEQ ID NO:815, SEQ ID NO:816, SEQ ID NO:817, SEQ ID NO:818, SEQ ID NO:819, SEQ ID NO:820, SEQ ID NO:821, SEQ ID NO:822, SEQ ID NO:823, SEQ ID NO:824, SEQ ID NO:825, SEQ ID NO:826, SEQ ID NO:827, SEQ ID NO:828, SEQ ID NO:829, SEQ ID NO:830, SEQ ID NO:831, SEQ ID NO:832, SEQ ID NO:833, SEQ ID NO:834, SEQ ID NO:835, SEQ ID NO:836, SEQ ID NO:837, SEQ ID NO:838, SEQ ID NO:839, SEQ ID NO:840, SEQ ID NO:841, SEQ ID NO:842, SEQ ID NO:843, SEQ ID NO:844, SEQ ID NO:845, SEQ ID NO:846, SEQ ID NO:847, SEQ ID NO:848, SEQ ID NO:849, SEQ ID NO:850, SEQ ID NO:851, SEQ ID NO:852, SEQ ID NO:853, SEQ ID NO:855, SEQ ID NO:856, SEQ ID NO:857, SEQ ID NO:858, SEQ ID NO:859, SEQ ID NO:860, SEQ ID NO:861, SEQ ID NO:862, SEQ ID NO:863, SEQ ID NO:864, SEQ ID NO:865, SEQ ID NO:866, SEQ ID NO:868, SEQ ID NO:869, SEQ ID NO:870, SEQ ID NO:871, SEQ ID NO:872, SEQ ID NO:873, SEQ ID NO:874, SEQ ID NO:875, SEQ ID NO:876, SEQ ID NO:877, SEQ ID NO:878, SEQ ID NO:879, SEQ ID NO:880, SEQ ID NO:881, SEQ ID NO:882, SEQ ID NO:883, SEQ ID NO:884, SEQ ID NO:885, SEQ ID NO:886, SEQ ID NO:887, SEQ ID NO:888, SEQ ID NO:889, SEQ ID NO:890, SEQ ID NO:891, SEQ ID NO:892, SEQ ID NO:893, SEQ ID NO:894, SEQ ID NO:895, SEQ ID NO:896, SEQ ID NO:897, SEQ ID NO:898, SEQ ID NO:899, SEQ ID NO:900, SEQ ID NO:901, SEQ ID NO:902, SEQ ID NO:903, SEQ ID NO:904, SEQ ID NO:905, SEQ ID NO:906, SEQ ID NO:907, SEQ ID NO:908, SEQ ID NO:909, SEQ ID NO:910, SEQ ID NO:911, SEQ ID NO:913, SEQ ID NO:914, SEQ ID NO:915, SEQ ID NO:916, SEQ ID NO:917, SEQ ID NO:918, SEQ ID NO:919, SEQ ID NO:920, SEQ ID NO:921, SEQ ID NO:923, SEQ ID NO:924, SEQ ID NO:925, SEQ ID NO:926, SEQ ID NO:927, SEQ ID NO:928, SEQ ID NO:929, SEQ ID NO:930, SEQ ID NO:931, SEQ ID NO:932, SEQ ID NO:933, SEQ ID NO:934, SEQ ID NO:935, SEQ ID NO:936, SEQ ID NO:937, SEQ ID NO:938, SEQ ID NO:939, SEQ ID NO:940, SEQ ID NO:941, SEQ ID NO:942, SEQ ID NO:943, SEQ ID NO:944, SEQ ID NO:945, SEQ ID NO:946, SEQ ID NO:947, SEQ ID NO:948, SEQ ID NO:949, SEQ ID NO:951, SEQ ID NO:952, SEQ ID NO:953, SEQ ID NO:954, SEQ ID NO:955, SEQ ID NO:956, SEQ ID NO:957, SEQ ID NO:958, SEQ ID NO:959, SEQ ID NO:960, SEQ ID NO:961, SEQ ID NO:962, SEQ ID NO:963, SEQ ID NO:964, SEQ ID NO:965, SEQ ID NO:966, SEQ ID NO:967, SEQ ID NO:968, SEQ ID NO:969, SEQ ID NO:970, SEQ ID NO:972, SEQ ID NO:973, SEQ ID NO:974, SEQ ID NO:975, SEQ ID NO:976, SEQ ID NO:977, SEQ ID NO:978, SEQ ID NO:979, SEQ ID NO:980, SEQ ID NO:981, SEQ ID NO:982, SEQ ID NO:983, SEQ ID NO:984, SEQ ID NO:985, SEQ ID NO:986, SEQ ID NO:987, SEQ ID NO:988, SEQ ID NO:989, SEQ ID NO:990, SEQ ID NO:991, SEQ ID NO:992, SEQ ID NO:993, SEQ ID NO:994, SEQ ID NO:995, SEQ ID NO:996, SEQ ID NO:997, SEQ ID NO:998, SEQ ID NO:999, SEQ ID NO:1000, SEQ ID NO:1001, SEQ ID NO:1002, SEQ ID NO:1003, SEQ ID NO:1004.

The methods of the invention may further include detecting the expression level of one or more additional genes in said patient to diagnose or monitor auto immune and chronic inflammatory diseases in a patient, wherein said one or more additional genes identified by a nucleotide sequence selected from the following group: SEQ ID NO:504, SEQ ID NO:507, SEQ ID NO:603, SEQ ID

NO:740, SEQ ID NO:811, SEQ ID NO:812, SEQ ID NO:854, SEQ ID NO:867, SEQ ID NO:912, SEQ ID NO:922, SEQ ID NO:950, SEQ ID NO:971.

In the method of the invention, the chronic inflammatory disease or autoimmune disease may be systemic lupus erythematosis (SLE), Rheumatoid Arthritis, Cholecystitis, Sjogrens Disease, CREST syndrome, Scleroderma, Ankylosing Spondylitis, Crohn's, Ulcerative Colitis, Primary Sclerosing Cholangitis, Appendicitis, Diverticulitis, Primary Biliary Sclerosis, Wegener's Granulomatosis, Polyarteritis nodosa, Whipple's Disease, Psoriasis, Microscopic Polyanngiitis, Takayasu's Disease, Kawasaki's Disease, Autoimmune hepatitis, Asthma, Churg-Strauss Disease, Beurger's Disease, Raynaud's Disease, or Cholecystitis.

In one format, expression is detecting by measuring RNA levels or protein levels from the genes. Example of detecting of such detection include measuring protein in serum, measuring cell surface proteins, measuring using a a fluorescent activated cell sorter.

In the method of the invention, RNA may be isolated from the patient prior to detecting expression of a gene such as those depicted in Table 2E. RNA levels may be detected by PCR or hybridization. The nucleotide sequence may include comprises DNA, cDNA, PNA, genomic DNA, or synthetic oligonucleotides. The hybridization methods of the present invention may include high stringency, moderate stringency, or low stringency hybridization conditions.

In the methods of the invention, the RNA may be detected by hybridization to an oligonucleotide having a nucleotide sequence selected from SEQ ID NO:503, SEQ ID NO:504, SEQ ID NO:505, SEQ ID NO:506, SEQ ID NO:507, SEQ ID NO:508, SEQ ID NO:509, SEQ ID NO:510, SEQ ID NO:511, SEQ ID NO:512, SEQ ID NO:513, SEQ ID NO:514, SEQ ID NO:515, SEQ ID NO:516, SEQ ID NO:517, SEQ ID NO:518, SEQ ID NO:519, SEQ ID NO:520, SEQ ID NO:521, SEQ ID NO:522, SEQ ID NO:523, SEQ ID NO:524, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:540, SEQ ID NO:541, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, SEQ ID NO:545, SEQ ID NO:546, SEQ ID NO:547, SEQ ID NO:548, SEQ ID NO:549, SEQ ID NO:550, SEQ ID NO:551, SEQ ID NO:552, SEQ ID NO:553, SEQ ID NO:554, SEQ ID NO:555, SEQ ID NO:556, SEQ ID NO:557, SEQ ID NO:558, SEQ ID NO:559, SEQ ID NO:560, SEQ ID NO:561, SEQ ID NO:562, SEQ ID NO:563, SEQ ID NO:564, SEQ ID NO:565, SEQ ID NO:566, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:569, SEQ ID NO:570, SEQ ID NO:571, SEQ ID NO:572, SEQ ID NO:573, SEQ ID NO:574, SEQ ID NO:575, SEQ ID NO:576, SEQ ID NO:577, SEQ ID NO:578, SEQ ID NO:579, SEQ ID NO:580, SEQ ID NO:581, SEQ ID NO:582, SEQ ID NO:583, SEQ ID NO:584, SEQ ID NO:585, SEQ ID NO:586, SEQ ID NO:587, SEQ ID NO:588, SEQ ID NO:589, SEQ ID NO:590, SEQ ID NO:591, SEQ ID NO:592, SEQ ID NO:593, SEQ ID NO:594, SEQ ID NO:595, SEQ ID NO:596, SEQ ID NO:597, SEQ ID NO:598, SEQ ID NO:599, SEQ ID NO:600, SEQ ID NO:601, SEQ ID NO:602, SEQ ID NO:603, SEQ ID NO:604, SEQ ID NO:605, SEQ ID NO:606, SEQ ID NO:607, SEQ ID NO:608, SEQ ID NO:609, SEQ ID NO:610, SEQ ID NO:611, SEQ ID NO:612, SEQ ID NO:613, SEQ ID NO:614, SEQ ID NO:615, SEQ ID NO:616, SEQ ID NO:617, SEQ ID NO:618, SEQ ID NO:619, SEQ ID NO:620, SEQ ID NO:621, SEQ ID NO:622, SEQ ID NO:623, SEQ

ID NO:624, SEQ ID NO:625, SEQ ID NO:626, SEQ ID NO:627, SEQ ID NO:628, SEQ ID NO:629, SEQ ID NO:630, SEQ ID NO:631, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:634, SEQ ID NO:635, SEQ ID NO:636, SEQ ID NO:637, SEQ ID NO:638, SEQ ID NO:639, SEQ ID NO:640, SEQ ID NO:641, SEQ ID NO:642, SEQ ID NO:643, SEQ ID NO:644, SEQ ID NO:645, SEQ ID NO:646, SEQ ID NO:647, SEQ ID NO:648, SEQ ID NO:649, SEQ ID NO:650, SEQ ID NO:651, SEQ ID NO:652, SEQ ID NO:653, SEQ ID NO:654, SEQ ID NO:655, SEQ ID NO:656, SEQ ID NO:657, SEQ ID NO:658, SEQ ID NO:659, SEQ ID NO:660, SEQ ID NO:661, SEQ ID NO:662, SEQ ID NO:663, SEQ ID NO:664, SEQ ID NO:665, SEQ ID NO:666, SEQ ID NO:667, SEQ ID NO:668, SEQ ID NO:669, SEQ ID NO:670, SEQ ID NO:671, SEQ ID NO:672, SEQ ID NO:673, SEQ ID NO:674, SEQ ID NO:675, SEQ ID NO:676, SEQ ID NO:677, SEQ ID NO:678, SEQ ID NO:679, SEQ ID NO:680, SEQ ID NO:681, SEQ ID NO:682, SEQ ID NO:683, SEQ ID NO:684, SEQ ID NO:685, SEQ ID NO:686, SEQ ID NO:687, SEQ ID NO:688, SEQ ID NO:689, SEQ ID NO:690, SEQ ID NO:691, SEQ ID NO:692, SEQ ID NO:693, SEQ ID NO:694, SEQ ID NO:695, SEQ ID NO:696, SEQ ID NO:697, SEQ ID NO:698, SEQ ID NO:699, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:705, SEQ ID NO:706, SEQ ID NO:707, SEQ ID NO:708, SEQ ID NO:709, SEQ ID NO:710, SEQ ID NO:711, SEQ ID NO:712, SEQ ID NO:713, SEQ ID NO:714, SEQ ID NO:715, SEQ ID NO:716, SEQ ID NO:717, SEQ ID NO:718, SEQ ID NO:719, SEQ ID NO:720, SEQ ID NO:721, SEQ ID NO:722, SEQ ID NO:723, SEQ ID NO:724, SEQ ID NO:725, SEQ ID NO:726, SEQ ID NO:727, SEQ ID NO:728, SEQ ID NO:729, SEQ ID NO:730, SEQ ID NO:731, SEQ ID NO:732, SEQ ID NO:733, SEQ ID NO:734, SEQ ID NO:735, SEQ ID NO:736, SEQ ID NO:737, SEQ ID NO:738, SEQ ID NO:739, SEQ ID NO:740, SEQ ID NO:741, SEQ ID NO:742, SEQ ID NO:743, SEQ ID NO:744, SEQ ID NO:745, SEQ ID NO:746, SEQ ID NO:747, SEQ ID NO:748, SEQ ID NO:749, SEQ ID NO:750, SEQ ID NO:751, SEQ ID NO:752, SEQ ID NO:753, SEQ ID NO:754, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID NO:759, SEQ ID NO:760, SEQ ID NO:761, SEQ ID NO:762, SEQ ID NO:763, SEQ ID NO:764, SEQ ID NO:765, SEQ ID NO:766, SEQ ID NO:767, SEQ ID NO:768, SEQ ID NO:769, SEQ ID NO:770, SEQ ID NO:771, SEQ ID NO:772, SEQ ID NO:773, SEQ ID NO:774, SEQ ID NO:775, SEQ ID NO:776, SEQ ID NO:777, SEQ ID NO:778, SEQ ID NO:779, SEQ ID NO:780, SEQ ID NO:781, SEQ ID NO:782, SEQ ID NO:783, SEQ ID NO:784, SEQ ID NO:785, SEQ ID NO:786, SEQ ID NO:787, SEQ ID NO:788, SEQ ID NO:789, SEQ ID NO:790, SEQ ID NO:791, SEQ ID NO:792, SEQ ID NO:793, SEQ ID NO:794, SEQ ID NO:795, SEQ ID NO:796, SEQ ID NO:797, SEQ ID NO:798, SEQ ID NO:799, SEQ ID NO:800, SEQ ID NO:801, SEQ ID NO:802, SEQ ID NO:803, SEQ ID NO:804, SEQ ID NO:805, SEQ ID NO:806, SEQ ID NO:807, SEQ ID NO:808, SEQ ID NO:809, SEQ ID NO:810, SEQ ID NO:811, SEQ ID NO:812, SEQ ID NO:813, SEQ ID NO:814, SEQ ID NO:815, SEQ ID NO:816, SEQ ID NO:817, SEQ ID NO:818, SEQ ID NO:819, SEQ ID NO:820, SEQ ID NO:821, SEQ ID NO:822, SEQ ID NO:823, SEQ ID NO:824, SEQ ID NO:825, SEQ ID NO:826, SEQ ID NO:827, SEQ ID NO:828, SEQ ID NO:829, SEQ ID NO:830, SEQ ID NO:831, SEQ ID NO:832, SEQ ID NO:833, SEQ ID NO:834, SEQ ID NO:835, SEQ ID NO:836, SEQ ID NO:837, SEQ ID NO:838, SEQ ID NO:839, SEQ ID NO:840, SEQ ID NO:841, SEQ ID NO:842, SEQ ID NO:843, SEQ ID NO:844, SEQ ID NO:845, SEQ ID NO:846, SEQ ID NO:847, SEQ ID NO:848, SEQ ID NO:849, SEQ ID NO:850,

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The methods of the present invention further includes detection of proteins expressed by one or more genes with an amino acid sequence encoded by a nucleotide sequence selected from the following group SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID

NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:165, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:216, SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:240, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, SEQ ID NO:257, SEQ ID NO:258, SEQ ID NO:259, SEQ ID NO:260, SEQ ID NO:261, SEQ ID NO:262, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:265, SEQ ID NO:266, SEQ ID NO:267, SEQ ID NO:268, SEQ ID NO:269, SEQ ID NO:270, SEQ ID NO:271, SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, SEQ ID NO:279, SEQ ID NO:280, SEQ ID NO:281, SEQ ID NO:282, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:285, SEQ ID NO:286, SEQ ID NO:287, SEQ ID NO:288, SEQ ID NO:289, SEQ ID NO:290, SEQ ID NO:291, SEQ ID NO:292, SEQ

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The methods of the present invention further include detection of one or more proteins expressed by one or more additional genes with an amino acid sequence encoded by a nucleotide sequence selected from the following group SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:101, SEQ ID

NO:238, SEQ ID NO:309, SEQ ID NO:310, SEQ ID NO:352, SEQ ID NO:365, SEQ ID NO:410, SEQ ID NO:420, SEQ ID NO:448, SEQ ID NO:469.

The present invention is further directed to a diagnostic oligonucleotide for detecting chronic or inflammatory disease wherein the oligonucleotide has a nucleotide sequence selected from SEQ ID NO:503, SEQ ID NO:504, SEQ ID NO:505, SEQ ID NO:506, SEQ ID NO:507, SEQ ID NO:508, SEQ ID NO:509, SEQ ID NO:510, SEQ ID NO:511, SEQ ID NO:512, SEQ ID NO:513, SEQ ID NO:514, SEQ ID NO:515, SEQ ID NO:516, SEQ ID NO:517, SEQ ID NO:518, SEQ ID NO:519, SEQ ID NO:520, SEQ ID NO:521, SEQ ID NO:522, SEQ ID NO:523, SEQ ID NO:524, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:540, SEQ ID NO:541, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, SEQ ID NO:545, SEQ ID NO:546, SEQ ID NO:547, SEQ ID NO:548, SEQ ID NO:549, SEQ ID NO:550, SEQ ID NO:551, SEQ ID NO:552, SEQ ID NO:553, SEQ ID NO:554, SEQ ID NO:555, SEQ ID NO:556, SEQ ID NO:557, SEQ ID NO:558, SEQ ID NO:559, SEQ ID NO:560, SEQ ID NO:561, SEQ ID NO:562, SEQ ID NO:563, SEQ ID NO:564, SEQ ID NO:565, SEQ ID NO:566, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:569, SEQ ID NO:570, SEQ ID NO:571, SEQ ID NO:572, SEQ ID NO:573, SEQ ID NO:574, SEQ ID NO:575, SEQ ID NO:576, SEQ ID NO:577, SEQ ID NO:578, SEQ ID NO:579, SEQ ID NO:580, SEQ ID NO:581, SEQ ID NO:582, SEQ ID NO:583, SEQ ID NO:584, SEQ ID NO:585, SEQ ID NO:586, SEQ ID NO:587, SEQ ID NO:588, SEQ ID NO:589, SEQ ID NO:590, SEQ ID NO:591, SEQ ID NO:592, SEQ ID NO:593, SEQ ID NO:594, SEQ ID NO:595, SEQ ID NO:596, SEQ ID NO:597, SEQ ID NO:598, SEQ ID NO:599, SEQ ID NO:600, SEQ ID NO:601, SEQ ID NO:602, SEQ ID NO:603, SEQ ID NO:604, SEQ ID NO:605, SEQ ID NO:606, SEQ ID NO:607, SEQ ID NO:608, SEQ ID NO:609, SEQ ID NO:610, SEQ ID NO:611, SEQ ID NO:612, SEQ ID NO:613, SEQ ID NO:614, SEQ ID NO:615, SEQ ID NO:616, SEQ ID NO:617, SEQ ID NO:618, SEQ ID NO:619, SEQ ID NO:620, SEQ ID NO:621, SEQ ID NO:622, SEQ ID NO:623, SEQ ID NO:624, SEQ ID NO:625, SEQ ID NO:626, SEQ ID NO:627, SEQ ID NO:628, SEQ ID NO:629, SEQ ID NO:630, SEQ ID NO:631, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:634, SEQ ID NO:635, SEQ ID NO:636, SEQ ID NO:637, SEQ ID NO:638, SEQ ID NO:639, SEQ ID NO:640, SEQ ID NO:641, SEQ ID NO:642, SEQ ID NO:643, SEQ ID NO:644, SEQ ID NO:645, SEQ ID NO:646, SEQ ID NO:647, SEQ ID NO:648, SEQ ID NO:649, SEQ ID NO:650, SEQ ID NO:651, SEQ ID NO:652, SEQ ID NO:653, SEQ ID NO:654, SEQ ID NO:655, SEQ ID NO:656, SEQ ID NO:657, SEQ ID NO:658, SEQ ID NO:659, SEQ ID NO:660, SEQ ID NO:661, SEQ ID NO:662, SEQ ID NO:663, SEQ ID NO:664, SEQ ID NO:665, SEQ ID NO:666, SEQ ID NO:667, SEQ ID NO:668, SEQ ID NO:669, SEQ ID NO:670, SEQ ID NO:671, SEQ ID NO:672, SEQ ID NO:673, SEQ ID NO:674, SEQ ID NO:675, SEQ ID NO:676, SEQ ID NO:677, SEQ ID NO:678, SEQ ID NO:679, SEQ ID NO:680, SEQ ID NO:681, SEQ ID NO:682, SEQ ID NO:683, SEQ ID NO:684, SEQ ID NO:685, SEQ ID NO:686, SEQ ID NO:687, SEQ ID NO:688, SEQ ID NO:689, SEQ ID NO:690, SEQ ID NO:691, SEQ ID NO:692, SEQ ID NO:693, SEQ ID NO:694, SEQ ID NO:695, SEQ ID NO:696, SEQ ID NO:697, SEQ ID NO:698, SEQ ID NO:699, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:705, SEQ ID NO:706, SEQ ID

NO:707, SEQ ID NO:708, SEQ ID NO:709, SEQ ID NO:710, SEQ ID NO:711, SEQ ID NO:712, SEQ ID NO:713, SEQ ID NO:714, SEQ ID NO:715, SEQ ID NO:716, SEQ ID NO:717, SEQ ID NO:718, SEQ ID NO:719, SEQ ID NO:720, SEQ ID NO:721, SEQ ID NO:722, SEQ ID NO:723, SEQ ID NO:724, SEQ ID NO:725, SEQ ID NO:726, SEQ ID NO:727, SEQ ID NO:728, SEQ ID NO:729, SEQ ID NO:730, SEQ ID NO:731, SEQ ID NO:732, SEQ ID NO:733, SEQ ID NO:734, SEQ ID NO:735, SEQ ID NO:736, SEQ ID NO:737, SEQ ID NO:738, SEQ ID NO:739, SEQ ID NO:740, SEQ ID NO:741, SEQ ID NO:742, SEQ ID NO:743, SEQ ID NO:744, SEQ ID NO:745, SEQ ID NO:746, SEQ ID NO:747, SEQ ID NO:748, SEQ ID NO:749, SEQ ID NO:750, SEQ ID NO:751, SEQ ID NO:752, SEQ ID NO:753, SEQ ID NO:754, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID NO:759, SEQ ID NO:760, SEQ ID NO:761, SEQ ID NO:762, SEQ ID NO:763, SEQ ID NO:764, SEQ ID NO:765, SEQ ID NO:766, SEQ ID NO:767, SEQ ID NO:768, SEQ ID NO:769, SEQ ID NO:770, SEQ ID NO:771, SEQ ID NO:772, SEQ ID NO:773, SEQ ID NO:774, SEQ ID NO:775, SEQ ID NO:776, SEQ ID NO:777, SEQ ID NO:778, SEQ ID NO:779, SEQ ID NO:780, SEQ ID NO:781, SEQ ID NO:782, SEQ ID NO:783, SEQ ID NO:784, SEQ ID NO:785, SEQ ID NO:786, SEQ ID NO:787, SEQ ID NO:788, SEQ ID NO:789, SEQ ID NO:790, SEQ ID NO:791, SEQ ID NO:792, SEQ ID NO:793, SEQ ID NO:794, SEQ ID NO:795, SEQ ID NO:796, SEQ ID NO:797, SEQ ID NO:798, SEQ ID NO:799, SEQ ID NO:800, SEQ ID NO:801, SEQ ID NO:802, SEQ ID NO:803, SEQ ID NO:804, SEQ ID NO:805, SEQ ID NO:806, SEQ ID NO:807, SEQ ID NO:808, SEQ ID NO:809, SEQ ID NO:810, SEQ ID NO:811, SEQ ID NO:812, SEQ ID NO:813, SEQ ID NO:814, SEQ ID NO:815, SEQ ID NO:816, SEQ ID NO:817, SEQ ID NO:818, SEQ ID NO:819, SEQ ID NO:820, SEQ ID NO:821, SEQ ID NO:822, SEQ ID NO:823, SEQ ID NO:824, SEQ ID NO:825, SEQ ID NO:826, SEQ ID NO:827, SEQ ID NO:828, SEQ ID NO:829, SEQ ID NO:830, SEQ ID NO:831, SEQ ID NO:832, SEQ ID NO:833, SEQ ID NO:834, SEQ ID NO:835, SEQ ID NO:836, SEQ ID NO:837, SEQ ID NO:838, SEQ ID NO:839, SEQ ID NO:840, SEQ ID NO:841, SEQ ID NO:842, SEQ ID NO:843, SEQ ID NO:844, SEQ ID NO:845, SEQ ID NO:846, SEQ ID NO:847, SEQ ID NO:848, SEQ ID NO:849, SEQ ID NO:850, SEQ ID NO:851, SEQ ID NO:852, SEQ ID NO:853, SEQ ID NO:854, SEQ ID NO:855, SEQ ID NO:856, SEQ ID NO:857, SEQ ID NO:858, SEQ ID NO:859, SEQ ID NO:860, SEQ ID NO:861, SEQ ID NO:862, SEQ ID NO:863, SEQ ID NO:864, SEQ ID NO:865, SEQ ID NO:866, SEQ ID NO:867, SEQ ID NO:868, SEQ ID NO:869, SEQ ID NO:870, SEQ ID NO:871, SEQ ID NO:872, SEQ ID NO:873, SEQ ID NO:874, SEQ ID NO:875, SEQ ID NO:876, SEQ ID NO:877, SEQ ID NO:878, SEQ ID NO:879, SEQ ID NO:880, SEQ ID NO:881, SEQ ID NO:882, SEQ ID NO:883, SEQ ID NO:884, SEQ ID NO:885, SEQ ID NO:886, SEQ ID NO:887, SEQ ID NO:888, SEQ ID NO:889, SEQ ID NO:890, SEQ ID NO:891, SEQ ID NO:892, SEQ ID NO:893, SEQ ID NO:894, SEQ ID NO:895, SEQ ID NO:896, SEQ ID NO:897, SEQ ID NO:898, SEQ ID NO:899, SEQ ID NO:900, SEQ ID NO:901, SEQ ID NO:902, SEQ ID NO:903, SEQ ID NO:904, SEQ ID NO:905, SEQ ID NO:906, SEQ ID NO:907, SEQ ID NO:908, SEQ ID NO:909, SEQ ID NO:910, SEQ ID NO:911, SEQ ID NO:912, SEQ ID NO:913, SEQ ID NO:914, SEQ ID NO:915, SEQ ID NO:916, SEQ ID NO:917, SEQ ID NO:918, SEQ ID NO:919, SEQ ID NO:920, SEQ ID NO:921, SEQ ID NO:922, SEQ ID NO:923, SEQ ID NO:924, SEQ ID NO:925, SEQ ID NO:926, SEQ ID NO:927, SEQ ID NO:928, SEQ ID NO:929, SEQ ID NO:930, SEQ ID NO:931, SEQ ID NO:932, SEQ ID NO:933, SEQ

ID NO:934, SEQ ID NO:935, SEQ ID NO:936, SEQ ID NO:937, SEQ ID NO:938, SEQ ID NO:939, SEQ ID NO:940, SEQ ID NO:941, SEQ ID NO:942, SEQ ID NO:943, SEQ ID NO:944, SEQ ID NO:945, SEQ ID NO:946, SEQ ID NO:947, SEQ ID NO:948, SEQ ID NO:949, SEQ ID NO:950, SEQ ID NO:951, SEQ ID NO:952, SEQ ID NO:953, SEQ ID NO:954, SEQ ID NO:955, SEQ ID NO:956, SEQ ID NO:957, SEQ ID NO:958, SEQ ID NO:959, SEQ ID NO:960, SEQ ID NO:961, SEQ ID NO:962, SEQ ID NO:963, SEQ ID NO:964, SEQ ID NO:965, SEQ ID NO:966, SEQ ID NO:967, SEQ ID NO:968, SEQ ID NO:969, SEQ ID NO:970, SEQ ID NO:971, SEQ ID NO:972, SEQ ID NO:973, SEQ ID NO:974, SEQ ID NO:975, SEQ ID NO:976, SEQ ID NO:977, SEQ ID NO:978, SEQ ID NO:979, SEQ ID NO:980, SEQ ID NO:981, SEQ ID NO:982, SEQ ID NO:983, SEQ ID NO:984, SEQ ID NO:985, SEQ ID NO:986, SEQ ID NO:987, SEQ ID NO:988, SEQ ID NO:989, SEQ ID NO:990, SEQ ID NO:991, SEQ ID NO:992, SEQ ID NO:993, SEQ ID NO:994, SEQ ID NO:995, SEQ ID NO:996, SEQ ID NO:997, SEQ ID NO:998, SEQ ID NO:999, SEQ ID NO:1000, SEQ ID NO:1001, SEQ ID NO:1002, SEQ ID NO:1003, SEQ ID NO:1004. The diagnostic oligonucleotide of may include DNA, cDNA, PNA, genomic DNA, or synthetic oligonucleotides.

The method of the present invention may further comprise selecting an appropriate therapy based upon the diagnosis and or monitoring. Such therapies may include administering appropriate drugs such as drugs that target alpha-interferon.

The methods of the present invention may be applied to bodily fluids from or in a patient, including peripheral blood and urine.

The present invention is further directed to a system or kit for diagnosing or monitoring chronic inflammatory or autoimmune disease in a patient comprising an isolated DNA molecule wherein the isolated DNA molecule detects expression of a gene listed in Table 2E. In the system of the invention, the DNA molecules may be synthetic DNA, genomic DNA, PNA or cDNA. The isolated DNA molecule may be immobilized on an array. Such arrays may include a chip array, a plate array, a bead array, a pin array, a membrane array, a solid surface array, a liquid array, an oligonucleotide array, polynucleotide array or a cDNA array, a microtiter plate, a membrane and a chip.

The present invention is further directed to a system or detecting differential gene expression. In one format, the system has one or more isolated DNA molecules wherein each isolated DNA molecule detects expression of a gene selected from the group of genes corresponding to the oligonucleotides depicted in the Sequence Listing. It is understood that the DNA sequences and oligonucleotides of the invention may have slightly different sequences than those identified herein. Such sequence variations are understood to those of ordinary skill in the art to be variations in the sequence which do not significantly affect the ability of the sequences to detect gene expression.

The sequences encompassed by the invention have at least 40-50, 50-60, 70-80, 80-85, 85-90, 90-95 or 95-100% sequence identity to the sequences disclosed herein. In some embodiments, DNA molecules are less than about any of the following lengths (in bases or base pairs): 10,000; 5,000; 2500; 2000; 1500; 1250; 1000; 750; 500; 300; 250; 200; 175; 150; 125; 100; 75; 50; 25; 10. In some embodiments, DNA molecule is greater than about any of the following lengths (in bases or base pairs): 10; 15; 20; 25; 30; 40; 50; 60; 75; 100; 125; 150; 175; 200; 250; 300; 350; 400; 500; 750; 1000; 2000; 5000; 7500; 10000; 20000; 50000. Alternately, a DNA molecule can be any of a range of sizes

having an upper limit of 10,000; 5,000; 2500; 2000; 1500; 1250; 1000; 750; 500; 300; 250; 200; 175; 150; 125; 100; 75; 50; 25; or 10 and an independently selected lower limit of 10; 15; 20; 25; 30; 40; 50; 60; 75; 100; 125; 150; 175; 200; 250; 300; 350; 400; 500; 750; 1000; 2000; 5000; 7500 wherein the lower limit is less than the upper limit.

The gene expression system may be a candidate library, a diagnostic agent, a diagnostic oligonucleotide set or a diagnostic probe set. The DNA molecules may be genomic DNA, protein nucleic acid (PNA), cDNA or synthetic oligonucleotides.

In one format, the gene expression system is immobilized on an array. The array may be a chip array, a plate array, a bead array, a pin array, a membrane array, a solid surface array, a liquid array, an oligonucleotide array, a polynucleotide array, a cDNA array, a microfilter plate, a membrane or a chip.

Brief Description of the Sequence Listing

A brief description of the sequence listing is given below. There are 1065 entries. The Sequence Listing presents 50mer oligonucleotide sequences derived from human leukocyte, plant and viral genes. These are listed as SEQ IDs 503-1004. The 50mer sequences and the corresponding gene sequences are also listed Table 2. Most of these 50mers were designed from sequences of genes in Table 2 and the Sequence listing.

SEQ ID's 1-502 represent mRNA sequences of genes those expression was altered in persons with SLE.

SEQ ID's 2-1004 are 50 nucleotide oligonucleotides used as probes to monitor RNA expression in blood.

SEQ ID's 1005-1037 are PCR primers and probes used to monitor expression of selected genes from 1-502

SEQ ID's 1038-1065 are sequences discussed in the Examples.

Brief Description of the Figures

Figure 1: Figure 1 is a schematic flow chart illustrating an instruction set for characterization of the nucleotide sequence and/or the predicted protein sequence of novel nucleotide sequences.

Figure 2: Figure 2 shows PCR Primer efficiency testing. A standard curve of Ct versus log of the starting RNA amount is shown for 2 genes.

Figure 3: Figure 3 describes kits useful for the practice of the invention. Figure 3A describes the contents of a kit useful for the discovery of diagnostic nucleotide sets using microarrays. Figure 3B describes the contents of a kit useful for the application of diagnostic nucleotide sets using microarrays. Figure 3C describes contents of a kit useful for the application of diagnostic nucleotide sets using real-time PCR.

Figure 4: Figure 4 depicts a graph comparing the median background subtracted expression signals for various leukocyte reference RNAs.

Figure 5: Figure 5 depicts Diagnostic genes, gene sets and diagnostic algorithms for Systemic Lupus Erythematosis are identified. **Figure 5A** shows the relative expression level of oligonucleotide and SEQ ID #16 (Sialyltransferase 4A) between Lupus and control samples is shown. The gene is

identified as having a false detection rate for differential expression from the SAM algorithm of 0.5%. **Figure 5B** shows the scaled ratios (non log) for Sialyltransferase (SEQ ID # 16) are given for the samples in the analysis. The average ratio of each group along with the standard deviation of the ratio is shown. The average fold change from control to Lupus is 1.48. **Figure 5C** shows CART gene expression models for diagnosis of SLE. For each model, the number of genes used, the relative cost with 10 fold cross validation, the SEQ ID, Locus accession number, the name and the position and values in the CART model are given. The CART values given are the expression level thresholds for classification of the sample as SLE after the node. For example, in the single gene model II, the first node of the decision tree asks if expression of gene SEQ ID NO 2 is >0.103. If yes, the sample is placed in the lupus class. **Figure 5D** shows the sensitivity and specificity of Model 1. The sensitivity and specificity are given for both the 2 and 3 gene models and both the training set and on cross validation. The relative cost is given for cross-validation. **Figure 5E** shows the CART Model I, 2 genes. The model uses 2 genes in a single node to classify samples as Lupus (Class 1) or non-Lupus (Class 2). **Figure 5F** shows CART Model I, 3 genes. The model uses a second node to classify all samples correctly as lupus (class 1) or non-lupus (class 2) for the training set. G2412 = SEQ ID 514, G2648 = SEQ ID 510, G1436 = SEQ ID 509.

Figure 6: Figure 6 shows endpoint testing of PCR primers. Electrophoresis and microfluidics are used to assess the product of gene specific PCR primers. **Figure 6A** is a β-GUS gel image. Lane 3 is the image for GUS primers. Lanes 2 and 1 correspond to the no-template control and –RT control, respectively. **Figure 6B** shows the electropherogram of β-GUS primers, a graphical representation of Lane 3 from the gel image. **Figure 6C** shows a β-Actin gel image. Lane 3 is the image for endpoint testing of actin primers. Lanes 2 and 1 correspond to the no-template control and –RT control, respectively. **Figure 6D** shows the electropherogram of β-Actin primers, a graphical representation of Lave 3 from the gel image.

Figure 7: Figure 7 shows the validation of differential expression of a gene discovered using microarrays using Real-time PCR. **Figure 7A** shows the Ct for each patient sample on multiple assays is shown along with the Ct in the R50 control RNA. Triangles represent –RT (reverse transcriptase) controls. **Figure 7B** shows the fold difference between the expression of Granzyme B and an Actin reference is shown for 3 samples from patients with and without CMV disease.

Figure 8: Real-time PCR control gene analysis. 11 candidate control genes were tested using real-time PCR on 6 whole blood samples (PAX) paired with 6 mononuclear samples (CPT) from the same patient. Each sample was tested twice. For each gene, the variability of the gene across the samples is shown on the vertical axis (top graph). The average Ct value for each gene is also shown (bottom graph). 2μg RNA was used for PAX samples and 0.5 μg total RNA was used for the mononuclear samples (CPT)

__Brief Description of the Tables__

Table 1: Samples used in array and PCR expression profiling experiments.

Samples were obtained from patients at a single medical center with appropriate IRB approval and informed consent. For each patient the primary clinical diagnosis is given according to American

College of Rheumatology criteria (SLE = Systemic Lupus Erythematosis, RA = Rheumatoid Arthritis, C = Healthy control, OA = Osteoarthritis). Dependent variables were defined for analysis from the patient clinical diagnoses. For Dx1, patients were classified as Lupus (1) or no Lupus (0). For Dx2, patients with either quiescent, uncertain or recently treated Lupus were removed from the analysis (2). PCR was done on the set of samples marked with an x.

Table 2: Gene expression markers for SLE and autoimmune disease

A: Significance analysis for Microarrays (SAM), Lupus/Autoimmune merkers. Each gene is identified by an oligonucleotide (SEQ ID 50 mer), Genbank accession number from VERSION (ACC), a full length (or longest known) RNA transcript (SEQ ID FL), and a unigene number VERSION (HS). Results for microarry analysis of blood gene expression (Example 11) are given as the false detection rate (SAM FDR) and a direction of expression change in Lupus patients / controls (SAM Up/Down).

B. Real-time PCR gene expression analysis. Real-time PCR was used to validate and quantify expression behavior of marker genes as described in Example 11. Each gene is identified by an oligonucleotide (SEQ ID 50 mer), Genbank accession number from VERSION (ACC), a full length (or longest known) RNA transcript (SEQ ID FL), and a unigene number VERSION (HS). The fold change between Lupus patients and controls (PCR fold) and results of an unpaired t-test (PCR p-value) are given.

C. Multiple Additive Regression Trees analysis of Microarray Data. The MART algorithm was used to identify marker genes and gene sets as described in Example 11. Each gene is identified by an oligonucleotide (SEQ ID 50 mer), Genbank accession number from VERSION (ACC), a full length (or longest known) RNA transcript (SEQ ID FL), and a unigene number VERSION (HS). The importance of the gene in the MART model (MART Importance), the error rate of the model that identified the gene (MART error) and the ratio of those 2 variables (Imp/error) are given.

D. Identification of pathways and pathway genes with hierarchical clustering. Genes are identified by close coexpression to significant genes from the microarray or PCR analysis (Hierarchical Cluster SEQ ID). This analysis identifies distinct pathways of gene expression.

Table 3: Table 3 lists some of the diseases or conditions amenable to study by leukocyte profiling.

Table 4: Real-time PCR assay reporter and quencher dyes. Various combinations of reporter and quencher dyes are useful for real-time PCR assays. Reporter and quencher dyes work optimally in specific combinations defined by their spectra. For each reporter, appropriate choices for quencher dyes are given.

Detailed Description of the Invention

Definitions

Unless defined otherwise, all scientific and technical terms are understood to have the same meaning as commonly used in the art to which they pertain. For the purpose of the present invention, the following terms are defined below.

In the context of the invention, the term "gene expression system" refers to any system, device or means to detect gene expression and includes diagnostic agents, candidate libraries oligonucleotide, oligonucleotide sets or probe sets.

The terms "diagnostic oligonucleotide" or "diagnostic oligonucleotide set" generally refers to an oligonucleotide or to a set of two or more oligonucleotides that, when evaluated for differential expression their corresponding diagnostic genes, collectively yields predictive data. Such predictive data typically relates to diagnosis, prognosis, monitoring of therapeutic outcomes, and the like. In general, the components of a diagnostic oligonucleotide or a diagnostic oligonucleotide set are distinguished from oligonucleotide sequences that are evaluated by analysis of the DNA to directly determine the genotype of an individual as it correlates with a specified trait or phenotype, such as a disease, in that it is the pattern of expression of the components of the diagnostic oligonucleotide set, rather than mutation or polymorphism of the DNA sequence that provides predictive value. It will be understood that a particular component (or member) of a diagnostic oligonucleotide set can, in some cases, also present one or more mutations, or polymorphisms that are amenable to direct genotyping by any of a variety of well known analysis methods, e.g., Southern blotting, RFLP, AFLP, SSCP, SNP, and the like.

A "diagnostic gene" is a gene whose expression is detected by a diagnostic oligonucleotide or diagnostic oligonucleotide set.

A "disease specific target oligonucleotide sequence" is a gene or other oligonucleotide that encodes a polypeptide, most typically a protein, or a subunit of a multi-subunit protein that is a therapeutic target for a disease, or group of diseases.

A "candidate library" or a "candidate oligonucleotide library" refers to a collection of oligonucleotide sequences (or gene sequences) that by one or more criteria have an increased probability of being associated with a particular disease or group of diseases. The criteria can be, for example, a differential expression pattern in a disease state or in activated or resting leukocytes in vitro as reported in the scientific or technical literature, tissue specific expression as reported in a sequence database, differential expression in a tissue or cell type of interest, or the like. Typically, a candidate library has at least 2 members or components; more typically, the library has in excess of about 10, or about 100, or about 1000, or even more, members or components.

The term "disease criterion" is used herein to designate an indicator of a disease, such as a diagnostic factor, a prognostic factor, a factor indicated by a medical or family history, a genetic factor, or a symptom, as well as an overt or confirmed diagnosis of a disease associated with several indicators such as those selected from the above list. A disease criterian includes data describing a patient's health status, including retrospective or prospective health data, e.g. in the form of the patient's medical history, laboratory test results, diagnostic test result, clinical events, medications, lists, response(s) to treatment and risk factors, etc.

An *autoimmune disorder* is defined as a disease state in which a patient's immune system recognizes an antigen in that patient's organs or tissues as foreign and becomes activated. The activated immune cells can then cause damage to the inciting organ or tissue or can damage other organs or tissues. In some cases, the disorder may be caused by a dysregulation of the immune system cells, rather than by the recognition as a self-antigen as foreign. Dysregulated immune cells can secrete inflammatory cytokines that cause systemic inflammation or they can recognize self-antigens as foreign.

Examples of autoimmune diseases include: Autoimmune hepatitis, Multiple Sclerosis, Myasthenia Gravis, Type I diabetes, Rheumatoid Arthritis, Psoriasis, Systemic Lupus Erythematosis, Hashimoto's Thyroiditis, Grave's disease, Ankylosing Spondylitis Sjogrens Disease, CREST syndrome, Scleroderma and many more.

Most of the autoimmune diseases are also *chronic inflammatory diseases*. This is defined as a disease process associated with long-term (>6 months) activation of inflammatory cells (leukocytes). The chronic inflammation leads to damage of patient organs or tissues. Many diseases are chronic inflammatory disorders, but are not know to have an autoimmune basis. For example, Atherosclerosis, Congestive Heart Failure, Crohn's disease, Ulcerative Colitis, Polyarteritis nodosa, Whipple's Disease, Primary Sclerosing Cholangitis and many more.

The terms "molecular signature" or "expression profile" refers to the collection of expression values for a plurality (e.g., at least 2, but frequently about 10, about 100, about 1000, or more) of members of a candidate library. In many cases, the molecular signature represents the expression pattern for all of the nucleotide sequences in a library or array of candidate or diagnostic nucleotide sequences or genes. Alternatively, the molecular signature represents the expression pattern for one or more subsets of the candidate library. The term "oligonucleotide" refers to two or more nucleotides. Nucleotides may be DNA or RNA, naturally occurring or synthetic.

The term "healthy individual," as used herein, is relative to a specified disease or disease criterion. That is, the individual does not exhibit the specified disease criterion or is not diagnosed with the specified disease. It will be understood, that the individual in question, can, of course, exhibit symptoms, or possess various indicator factors for another disease.

Similarly, an "individual diagnosed with a disease" refers to an individual diagnosed with a specified disease (or disease criterion). Such an individual may, or may not, also exhibit a disease criterion associated with, or be diagnosed with another (related or unrelated) disease.

The term "monitoring" is used herein to describe the use of gene sets to provide useful information about an individual or an individual's health or disease status. "Monitoring" can include, determination of prognosis, risk-stratification, selection of drug therapy, assessment of ongoing drug therapy, prediction of outcomes, determining response to therapy, diagnosis of a disease or disease complication, following progression of a disease or providing any information relating to a patients health status over time, selecting patients most likely to benefit from experimental therapies with known molecular mechanisms of action, selecting patients most likely to benefit from approved drugs with known molecular mechanisms where that mechanism may be important in a small subset of a disease for which the medication may not have a label, screening a patient population to help decide on a more invasive/expensive test, for example a cascade of tests from a non-invasive blood test to a more invasive option such as biopsy, or testing to assess side effects of drugs used to treat another indication..

An "array" is a spatially or logically organized collection, e.g., of oligonucleotide sequences or nucleotide sequence products such as RNA or proteins encoded by an oligonucleotide sequence. In some embodiments, an array includes antibodies or other binding reagents specific for products of a candidate library.

When referring to a pattern of expression, a "qualitative" difference in gene expression refers to a difference that is not assigned a relative value. That is, such a difference is designated by an "all or nothing" valuation. Such an all or nothing variation can be, for example, expression above or below a threshold of detection (an on/off pattern of expression). Alternatively, a qualitative difference can refer to expression of different types of expression products, e.g., different alleles (e.g., a mutant or polymorphic allele), variants (including sequence variants as well as post-translationally modified variants), etc.

In contrast, a "quantitative" difference, when referring to a pattern of gene expression, refers to a difference in expression that can be assigned a value on a graduated scale, (e.g., a 0-5 or 1-10 scale, a + - +++ scale, a grade 1- grade 5 scale, or the like; it will be understood that the numbers selected for illustration are entirely arbitrary and in no-way are meant to be interpreted to limit the invention).

Gene Expression Systems and Methods of Detecting Gene Expression

The invention is directed to methods of detecting gene expression with a gene expression system having one or more DNA molecules wherein the one or more DNA molecules has a nucleotide sequence which detects expression of a gene corresponding to the oligonucleotides depicted in the Sequence Listing. In one format, the oligonucleotide detects expression of a gene that is differentially expressed in leukocytes. The gene expression system may be a candidate library, a diagnostic agent, a diagnostic oligonucleotide set or a diagnostic probe set. The DNA molecules may be genomic DNA, RNA, protein nucleic acid (PNA), cDNA or synthetic oligonucleotides. Following the procedures taught herein, one can identity sequences of interest for analyzing gene expression in leukocytes. Such sequences may be predictive of a disease state.

Diagnostic oligonucleotides of the invention

The invention relates to diagnostic oligonucleotides and diagnostic oligonucleotide set(s) comprising members of the leukocyte candidate library listed in Table 2 and the Sequence Listing, for which a correlation exists between the health status of an individual, and the individual's expression of RNA or protein products corresponding to the nucleotide sequence. In some instances, only one oligonucleotide is necessary for such detection. Members of a diagnostic oligonucleotide set may be identified by any means capable of detecting expression of RNA or protein products, including but not limited to differential expression screening, PCR, RT-PCR, SAGE analysis, high-throughput sequencing, microarrays, liquid or other arrays, protein-based methods (e.g., western blotting, proteomics, and other methods described herein), and data mining methods, as further described herein.

In one embodiment, a diagnostic oligonucleotide set comprises at least two oligonucleotide sequences listed in Table 2 or the Sequence Listing which are differentially expressed in leukocytes in an individual with at least one disease criterion for at least one leukocyte-implicated disease relative to the expression in individual without the at least one disease criterion, wherein expression of the two or more nucleotide sequences is correlated with at least one disease criterion, as described below.

In another embodiment, a diagnostic oligonucleotide set comprises at least one oligonucleotide having an oligonucleotide sequence listed in Table 2 or the Sequence Listing which is

differentially expressed, and further wherein the differential expression/correlation has not previously been described. In some embodiments, the diagnostic oligonucleotide set is immobilized on an array.

In another embodiment, diagnostic oligonucleotides (or oligonucleotide sets) are related to the members of the leukocyte candidate library listed in Table 2 and in the Sequence Listing, for which a correlation exists between the health status (or disease criterion) of an individual. The diagnostic oligonucleotides are partially or totally contained in (or derived from) full-length gene sequences (or predicted full-length gene sequences) for the members of the candidate library listed in Table 2 and the Sequence Listing.

The diagnostic oligonucleotides may also be derived from other genes that are coexpressed with the correlated sequence or full-length gene. Genes may share expression patterns because they are regulated in the same molecular pathway. Because of the similarity of expression, behavior genes are identified as surrogates in that they can substitute for a diagnostic gene in a diagnostic gene set. Example 4 demonstrates the discovery of surrogates from the data. Surrogate oligonucleotide and surrogate oligonucleotide sets can be utilized to detect expression of surrogate genes and thereby diagnose or monitor patients with a disease.

As used herein the term "gene cluster" or "cluster" refers to a group of genes related by expression pattern. In other words, a cluster of genes is a group of genes with similar regulation across different conditions, such as a patient having a chronic autoimmune or inflammatory disease or a patient without chronic autoimmune or inflammatory disease. The expression profile for each gene in a cluster should be correlated with the expression profile of at least one other gene in that cluster. Correlation may be evaluated using a variety of statistical methods. As used herein the term "surrogate" refers to a gene with an expression profile such that it can substitute for a diagnostic gene in a diagnostic assay. Such genes are often members of the same gene cluster as the diagnostic gene. For each member of a diagnostic gene set, a set of potential surrogates can be identified through identification of genes with similar expression patterns as described below.

Many statistical analyses produce a correlation coefficient to describe the relatedness between two gene expression patterns. Patterns may be considered correlated if the correlation coefficient is greater than or equal to 0.8. In preferred embodiments, the correlation coefficient should be greater than 0.85, 0.9 or 0.95. Other statistical methods produce a measure of mutual information to describe the relatedness between two gene expression patterns. Patterns may be considered correlated if the normalized mutual information value is greater than or equal to 0.7. In preferred embodiments, the normalized mutual information value should be greater than 0.8, 0.9 or 0.95. Patterns may also be considered similar if they cluster closely upon hierarchical clustering of gene expression data (Eisen et al. 1998). Similar patterns may be those genes that are among the 1, 2, 5, 10, 20, 50 or 100 nearest neighbors in a hierarchical clustering or have a similarity score (Eisen et al. 1998) of > 0.5, 0.7, 0.8, 0.9, 0.95 or 0.99. Similar patterns may also be identified as those genes found to be surrogates in a classification tree by CART (Breiman et al. 1994). Often, but not always, members of a gene cluster have similar biological functions in addition to similar gene expression patterns.

Correlated genes, clusters and surrogates are identified for the diagnostic genes of the invention. These surrogates may be used as diagnostic genes in an assay instead of, or in addition to, the diagnostic genes for which they are surrogates.

The invention also provides diagnostic probe sets. It is understood that a probe includes any reagent capable of specifically identifying a nucleotide sequence of the diagnostic nucleotide set, including but not limited to amplified DNA, amplified RNA, cDNA, synthetic oligonucleotide, partial or full-length nucleic acid sequences. In addition, the probe may identify the protein product of a diagnostic nucleotide sequence, including, for example, antibodies and other affinity reagents.

It is also understood that each probe can correspond to one gene, or multiple probes can correspond to one gene, or both, or one probe can correspond to more than one gene.

Homologs and variants of the disclosed nucleic acid molecules may be used in the present invention. Homologs and variants of these nucleic acid molecules will possess a relatively high degree of sequence identity when aligned using standard methods. The sequences encompassed by the invention have at least 40-50, 50-60, 70-80, 80-85, 85-90, 90-95 or 95-100% sequence identity to the sequences disclosed herein.

It is understood that for expression profiling, variations in the disclosed sequences will still permit detection of gene expression. The degree of sequence identity required to detect gene expression varies depending on the length of the oligomer. For a 60 mer, (an oligonucleotide with 60 nucleotides) 6-8 random mutations or 6-8 random deletions in a 60 mer do not affect gene expression detection. Hughes, TR, et al. "Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. Nature Biotechnology, 19:343-347(2001). As the length of the DNA sequence is increased, the number of mutations or deletions permitted while still allowing gene expression detection is increased.

As will be appreciated by those skilled in the art, the sequences of the present invention may contain sequencing errors. That is, there may be incorrect nucleotides, frameshifts, unknown nucleotides, or other types of sequencing errors in any of the sequences; however, the correct sequences will fall within the homology and stringency definitions herein.

The minimum length of an oligonucleotide probe necessary for specific hybridization in the human genome can be estimated using two approaches. The first method uses a statistical argument that the probe will be unique in the human genome by chance. Briefly, the number of independent perfect matches (Po) expected for an oligonucleotide of length L in a genome of complexity C can be calculated from the equation (Laird CD, Chromosoma 32:378 (1971):

$$Po=(1/4)^L * 2C$$

In the case of mammalian genomes, $2C = \sim 3.6 \times 10^9$, and an oligonucleotide of 14-15 nucleotides is expected to be represented only once in the genome. However, the distribution of nucleotides in the coding sequence of mammalian genomes is nonrandom (Lathe, R. J. Mol. Biol. 183:1 (1985) and longer oligonucleotides may be preferred in order to in increase the specificity of hybridization. In practical terms, this works out to probes that are 19-40 nucleotides long (Sambrook J et al., infra). The second method for estimating the length of a specific probe is to use a probe long enough to hybridize under the chosen conditions and use a computer to search for that sequence or

close matches to the sequence in the human genome and choose a unique match. Probe sequences are chosen based on the desired hybridization properties as described in Chapter 11 of Sambrook et al, infra. The PRIMER3 program is useful for designing these probes (S. Rozen and H. Skaletsky 1996,1997; Primer3 code available at genome.wi.mit.edu/genome_software/other/primer3.html, the website). The sequences of these probes are then compared pair wise against a database of the human genome sequences using a program such as BLAST or MEGABLAST (Madden, T.L et al.(1996) Meth. Enzymol. 266:131-141). Since most of the human genome is now contained in the database, the number of matches will be determined. Probe sequences are chosen that are unique to the desired target sequence.

In some embodiments, a diagnostic oligonucleotide or oligonucleotide probe set is immobilized on an array. The array is optionally comprises one or more of: a chip array, a plate array, a bead array, a pin array, a membrane array, a solid surface array, a liquid array, an oligonucleotide array, a polynucleotide array or a cDNA array, a microtiter plate, a pin array, a bead array, a membrane or a chip.

In some embodiments, the leukocyte-implicated disease is selected from the diseases listed in Table 3. In other embodiments, the disease is chronic autoimmune and inflammatory diseases, systemic lupus erythematosis (SLE) and rheumatoid arthritis.

In some embodiments, diagnostic oligonucleotides of the invention are used as a diagnostic gene set in combination with genes that are know to be associated with a disease state ("known markers"). The use of the diagnostic oligonucleotides in combination with the known markers can provide information that is not obtainable through the known markers alone. The known markers include those identified by the prior art listing provided.

General Molecular Biology References

In the context of the invention, nucleic acids and/or proteins are manipulated according to well known molecular biology techniques. Detailed protocols for numerous such procedures are described in, e.g., in Ausubel et al. Current Protocols in Molecular Biology (supplemented through 2000) John Wiley & Sons, New York ("Ausubel"); Sambrook et al. Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook"), and Berger and Kimmel Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA ("Berger").

In addition to the above references, protocols for in vitro amplification techniques, such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), Q-replicase amplification, and other RNA polymerase mediated techniques (e.g., NASBA), useful e.g., for amplifying cDNA probes of the invention, are found in Mullis et al. (1987) U.S. Patent No. 4,683,202; PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) ("Innis"); Arnheim and Levinson (1990) C&EN 36; The Journal Of NIH Research (1991) 3:81; Kwoh et al. (1989) Proc Natl Acad Sci USA 86, 1173; Guatelli et al. (1990) Proc Natl Acad Sci USA 87:1874; Lomell et al. (1989) J Clin Chem 35:1826; Landegren et al. (1988) Science 241:1077; Van Brunt (1990) Biotechnology 8:291; Wu and Wallace (1989) Gene 4: 560; Barringer et al. (1990) Gene 89:117, and Sooknanan and Malek (1995) Biotechnology 13:563. Additional methods, useful for

cloning nucleic acids in the context of the present invention, include Wallace et al. U.S. Pat. No. 5,426,039. Improved methods of amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369:684 and the references therein.

Certain polynucleotides of the invention, e.g., oligonucleotides can be synthesized utilizing various solid-phase strategies involving mononucleotide- and/or trinucleotide-based phosphoramidite coupling chemistry. For example, nucleic acid sequences can be synthesized by the sequential addition of activated monomers and/or trimers to an elongating polynucleotide chain. See e.g., Caruthers, M.H. et al. (1992) Meth Enzymol 211:3.

In lieu of synthesizing the desired sequences, essentially any nucleic acid can be custom ordered from any of a variety of commercial sources, such as The Midland Certified Reagent Company (mcrc@oligos.com), The Great American Gene Company (genco.com), ExpressGen, Inc. (expressgen.com), Operon Technologies, Inc. (operon.com), and many others.

Similarly, commercial sources for nucleic acid and protein microarrays are available, and include, e.g., Agilent Technologies, Palo Alto, CA and Affymetrix, Santa Clara,CA.

One area of relevance to the present invention is hybridization of oligonucleotides. Those of skill in the art differentiate hybridization conditions based upon the stringency of hybridization. For example, highly stringent conditions could include hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65° C, and washing in 0.1XSSC/0.1% SDS at 68° C. (Ausubel F. M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3). Moderate stringency conditions could include, e.g., washing in 0.2XSSC/0.1% SDS at 42°C. (Ausubel et al., 1989, supra).

The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the DNA sequences of the present invention. Such hybridization conditions may be highly stringent or less highly stringent, as described above. In instances wherein the nucleic acid molecules are deoxyoligonucleotides ("oligos"), highly stringent conditions may refer, e.g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C. (for 14-base oligos), 48°C. (for 17-base oligos), 55°C. (for 20-base oligos), and 60°C. (for 23-base oligos). These nucleic acid molecules may act as target nucleotide sequence antisense molecules, useful, for example, in target nucleotide sequence regulation and/or as antisense primers in amplification reactions of target nucleotide sequence nucleic acid sequences. Further, such sequences may be used as part of ribozyme and/or triple helix sequences, also useful for target nucleotide sequence regulation. Still further, such molecules may be used as components of diagnostic methods whereby the presence of a disease-causing allele, may be detected.

Candidate library

Libraries of candidate genes that are differentially expressed in leukocytes are substrates for the identification and evaluation of diagnostic oligonucleotides and oligonucleotide sets and disease specific target nucleotide sequences.

The term leukocyte is used generically to refer to any nucleated blood cell that is not a nucleated erythrocyte. More specifically, leukocytes can be subdivided into two broad classes. The

first class includes granulocytes, including, most prevalently, neutrophils, as well as eosinophils and basophils at low frequency. The second class, the non-granular or mononuclear leukocytes, includes monocytes and lymphocytes (e.g., T cells and B cells). There is an extensive literature in the art implicating leukocytes, e.g., neutrophils, monocytes and lymphocytes in a wide variety of disease processes, including inflammatory and rheumatic diseases, neurodegenerative diseases (such as Alzheimer's dementia), cardiovascular disease, endocrine diseases, transplant rejection, malignancy and infectious diseases, and other diseases listed in Table 3. Mononuclear cells are involved in the chronic immune response, while granulocytes, which make up approximately 60% of the leukocytes, have a non-specific and stereotyped response to acute inflammatory stimuli and often have a life span of only 24 hours.

In addition to their widespread involvement and/or implication in numerous disease related processes, leukocytes are particularly attractive substrates for clinical and experimental evaluation for a variety of reasons. Most importantly, they are readily accessible at low cost from essentially every potential subject. Collection is minimally invasive and associated with little pain, disability or recovery time. Collection can be performed by minimally trained personnel (e.g., phlebotomists, medical technicians, etc.) in a variety of clinical and non-clinical settings without significant technological expenditure. Additionally, leukocytes are renewable, and thus available at multiple time points for a single subject.

Assembly of an initial candidate library

The initial candidate library was assembled from a combination of "mining" publication and sequence databases and construction of a differential expression library. Candidate oligonucleotide sequences in the library may be represented by a full-length or partial nucleic acid sequence, deoxyribonucleic acid (DNA) sequence, cDNA sequence, RNA sequence, synthetic oligonucleotides, etc. The nucleic acid sequence can be at least 19 nucleotides in length, at least 25 nucleotides, at least 40 nucleotides, at least 100 nucleotides, or larger. Alternatively, the protein product of a candidate nucleotide sequence may be represented in a candidate library using standard methods, as further described below. In selecting and validatating diagnostic oligonucleotides, an initial library of 8,031 candidate oligonucleotide sequences using nucleic acid sequences of 50 nucleotides in length was constructed as described below.

Candidate nucleotide library

We identified members of an initial candidate nucleotide library that are differentially expressed in activated leukocytes and resting leukocytes. From that initial candidate nucleotide library, a pool of 502 candidates were selected. Accordingly, the invention provides the candidate leukocyte nucleotide library comprising the nucleotide sequences listed in Table 2 and in the Sequence Listing. In another embodiment, the invention provides a candidate library comprising at least two nucleotide sequences listed in Table 2 and the Sequence Listing. In another embodiment, at least two nucleotide sequences are 18 nucleotides in length, at least 35 nucleotides, at least 40 nucleotides or at least 100 nucleotides. In some embodiments, the nucleotide sequences comprises deoxyribonucleic acid (DNA) sequence, ribonucleic acid (RNA) sequence, synthetic oligonucleotide sequence, or genomic DNA

sequence. It is understood that the nucleotide sequences may each correspond to one gene, or that several nucleotide sequences may correspond to one gene, or that a single nucleotide sequence may correspond to multiple genes.

The invention also provides probes to the candidate nucleotide library. In one embodiment of the invention, the probes comprise at least two nucleotide sequences listed in Table 2 or the Sequence Listing which are differentially expressed in leukocytes in an individual with a least one disease criterion for at least one leukocyte-related disease and in leukocytes in an individual without the at least one disease criterion, wherein expression of the two or more nucleotide sequences is correlated with at least one disease criterion. It is understood that a probe may detect either the RNA expression or protein product expression of the candidate nucleotide library. Alternatively, or in addition, a probe can detect a genotype associated with a candidate nucleotide sequence, as further described below. In another embodiment, the probes for the candidate nucleotide library are immobilized on an array.

The candidate nucleotide library of the invention is useful in identifying diagnostic nucleotide sets of the invention and is itself a diagnostic nucleotide set of the invention, as described below. The candidate nucleotide sequences may be further characterized, and may be identified as a disease target nucleotide sequence, as described below. The candidate nucleotide sequences may also be suitable for use as imaging reagents, as described below.

Generation of Expression Patterns

<u>RNA, DNA or protein sample procurement</u>

Following identification or assembly of a library of differentially expressed candidate nucleotide sequences, leukocyte expression profiles corresponding to multiple members of the candidate library are obtained. Leukocyte samples from one or more subjects are obtained by standard methods. Most typically, these methods involve trans-cutaneous venous sampling of peripheral blood. While sampling of circulating leukocytes from whole blood from the peripheral vasculature is generally the simplest, least invasive, and lowest cost alternative, it will be appreciated that numerous alternative sampling procedures exist, and are favorably employed in some circumstances. No pertinent distinction exists, in fact, between leukocytes sampled from the peripheral vasculature, and those obtained, e.g., from a central line, from a central artery, or indeed from a cardiac catheter, or during a surgical procedure which accesses the central vasculature. In addition, other body fluids and tissues that are, at least in part, composed of leukocytes are also desirable leukocyte samples. For example, fluid samples obtained from the lung during bronchoscopy may be rich in leukocytes, and amenable to expression profiling in the context of the invention, e.g., for the diagnosis, prognosis, or monitoring of lung transplant rejection, inflammatory lung diseases or infectious lung disease. Fluid samples from other tissues, e.g., obtained by endoscopy of the colon, sinuses, esophagus, stomach, small bowel, pancreatic duct, biliary tree, bladder, ureter, vagina, cervix or uterus, etc., are also suitable. Samples may also be obtained other sources containing leukocytes, e.g., from urine, bile, cerebrospinal fluid, feces, gastric or intestinal secretions, semen, or solid organ or joint biopsies.

Most frequently, mixed populations of leukocytes, such as are found in whole blood are utilized in the methods of the present invention. A crude separation, e.g., of mixed leukocytes from red blood cells, and/or concentration, e.g., over a sucrose, percoll or ficoll gradient, or by other methods

known in the art, can be employed to facilitate the recovery of RNA or protein expression products at sufficient concentrations, and to reduce non-specific background. In some instances, it can be desirable to purify sub-populations of leukocytes, and methods for doing so, such as density or affinity gradients, flow cytometry, Fluorescence Activated Cell Sorting (FACS), immuno-magnetic separation, "panning," and the like, are described in the available literature and below.

Obtaining DNA, RNA and protein samples for expression profiling

A variety of techniques are available for the isolation of RNA from whole blood. Any technique that allows isolation of mRNA from cells (in the presence or absence of rRNA and tRNA) can be utilized. In brief, one method that allows reliable isolation of total RNA suitable for subsequent gene expression analysis is described as follows. Peripheral blood (either venous or arterial) is drawn from a subject, into one or more sterile, endotoxin free, tubes containing an anticoagulant (e.g., EDTA, citrate, heparin, etc.). Typically, the sample is divided into at least two portions. One portion, e.g., of 5-8 ml of whole blood is frozen and stored for future analysis, e.g., of DNA or protein. A second portion, e.g., of approximately 8 ml whole blood is processed for isolation of total RNA by any of a variety of techniques as described in, e.g, Sambook, Ausubel, below, as well as U.S. Patent Numbers: 5,728,822 and 4,843,155.

Typically, a subject sample of mononuclear leukocytes obtained from about 8 ml of whole blood, a quantity readily available from an adult human subject under most circumstances, yields 5-20 μg of total RNA. This amount is ample, e.g., for labeling and hybridization to at least two probe arrays. Labeled probes for analysis of expression patterns of nucleotides of the candidate libraries are prepared from the subject's sample of RNA using standard methods. In many cases, cDNA is synthesized from total RNA using a polyT primer and labeled, e.g., radioactive or fluorescent, nucleotides. The resulting labeled cDNA is then hybridized to probes corresponding to members of the candidate nucleotide library, and expression data is obtained for each nucleotide sequence in the library. RNA isolated from subject samples (e.g., peripheral blood leukocytes, or leukocytes obtained from other biological fluids and samples) is next used for analysis of expression patterns of nucleotides of the candidate libraries.

In some cases, however, the amount of RNA that is extracted from the leukocyte sample is limiting, and amplification of the RNA is desirable. Amplification may be accomplished by increasing the efficiency of probe labeling, or by amplifying the RNA sample prior to labeling. It is appreciated that care must be taken to select an amplification procedure that does not introduce any bias (with respect to gene expression levels) during the amplification process.

Several methods are available that increase the signal from limiting amounts of RNA, e.g. use of the Clontech (Glass Fluorescent Labeling Kit) or Stratagene (Fairplay Microarray Labeling Kit), or the Micromax kit (New England Nuclear, Inc.). Alternatively, cDNA is synthesized from RNA using a T7- polyT primer, in the absence of label, and DNA dendrimers from Genisphere (3DNA Submicro) are hybridized to the poly T sequence on the primer, or to a different "capture sequence" which is complementary to a fluorescently labeled sequence. Each 3DNA molecule has 250 fluorescent molecules and therefore can strongly label each cDNA.

Alternatively, the RNA sample is amplified prior to labeling. For example, linear amplification may be performed, as described in U.S. Patent No. 6,132,997. A T7-polyT primer is used to generate the cDNA copy of the RNA. A second DNA strand is then made to complete the substrate for amplification. The T7 promoter incorporated into the primer is used by a T7 polymerase to produce numerous antisense copies of the original RNA. Fluorescent dye labeled nucleotides are directly incorporated into the RNA. Alternatively, amino allyl labeled nucleotides are incorporated into the RNA, and then fluorescent dyes are chemically coupled to the amino allyl groups, as described in Hughes et al. 2001. Other exemplary methods for amplification are described below.

It is appreciated that the RNA isolated must contain RNA derived from leukocytes, but may also contain RNA from other cell types to a variable degree. Additionally, the isolated RNA may come from subsets of leukocytes, e.g. monocytes and/or T-lymphocytes, as described above. Such consideration of cell type used for the derivation of RNA depends on the method of expression profiling used. Subsets of leukocytes can be obtained by fluorescence activated cell sorting (FACS), microfluidics cell seperation systems or a variety of other methods. Cell sorting may be necessary for the discovery of diagnostic gene sets, for the implementation of gene sets as products or both. Cell sorting can be achieved with a variety of technologies (See Galbraith et al. 1999, Cantor et al. 1975, see also the technology of Guava Technologies, Hayward, CA).

DNA samples may be obtained for analysis of the presence of DNA mutations, single nucleotide polymorphisms (SNPs), or other polymorphisms. DNA is isolated using standard techniques, e.g. *Maniatus, supra*.

Expression of products of candidate nucleotides may also be assessed using proteomics. Protein(s) are detected in samples of patient serum or from leukocyte cellular protein. Serum is prepared by centrifugation of whole blood, using standard methods. Proteins present in the serum may have been produced from any of a variety of leukocytes and non-leukocyte cells, and may include secreted proteins from leukocytes. Alternatively, leukocytes or a desired sub-population of leukocytes are prepared as described above. Cellular protein is prepared from leukocyte samples using methods well known in the art, e.g., Trizol (Invitrogen Life Technologies, cat # 15596108; Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156; Simms, D., Cizdziel, P.E., and Chomczynski, P. (1993) Focus® 15, 99; Chomczynski, P., Bowers-Finn, R., and Sabatini, L. (1987) J. of NIH Res. 6, 83; Chomczynski, P. (1993) Bio/Techniques 15, 532; Bracete, A.M., Fox, D.K., and Simms, D. (1998) Focus 20, 82; Sewall, A. and McRae, S. (1998) Focus 20, 36; Anal Biochem 1984 Apr;138(1):141-3, A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids; Wessel D, Flugge UI. (1984) Anal Biochem. 1984 Apr;138(1):141-143.

The assay itself may be a cell sorting assay in which cells are sorted and/or counted based on cell surface expression of a protein marker. (See Cantor et al. 1975, Galbraith et al. 1999)

Obtaining expression patterns

Expression patterns, or profiles, of a plurality of nucleotides corresponding to members of the candidate library are then evaluated in one or more samples of leukocytes. Typically, the leukocytes are derived from patient peripheral blood samples, although, as indicated above, many other sample sources are also suitable. These expression patterns constitute a set of relative or absolute expression

values for some number of RNAs or protein products corresponding to the plurality of nucleotide sequences evaluated, which is referred to herein as the subject's "expression profile" for those nucleotide sequences. While expression patterns for as few as one independent member of the candidate library can be obtained, it is generally preferable to obtain expression patterns corresponding to a larger number of nucleotide sequences, e.g., about 2, about 5, about 10, about 20, about 50, about 100, about 200, about 500, or about 1000, or more. The expression pattern for each differentially expressed component member of the library provides a finite specificity and sensitivity with respect to predictive value, e.g., for diagnosis, prognosis, monitoring, and the like.

Clinical Studies, Data and Patient Groups

For the purpose of discussion, the term subject, or subject sample of leukocytes, refers to an individual regardless of health and/or disease status. A subject can be a patient, a study participant, a control subject, a screening subject, or any other class of individual from whom a leukocyte sample is obtained and assessed in the context of the invention. Accordingly, a subject can be diagnosed with a disease, can present with one or more symptom of a disease, or a predisposing factor, such as a family (genetic) or medical history (medical) factor, for a disease, or the like. Alternatively, a subject can be healthy with respect to any of the aforementioned factors or criteria. It will be appreciated that the term "healthy" as used herein, is relative to a specified disease, or disease factor, or disease criterion, as the term "healthy" cannot be defined to correspond to any absolute evaluation or status. Thus, an individual defined as healthy with reference to any specified disease or disease criterion, can in fact be diagnosed with any other one or more disease, or exhibit any other one or more disease criterion.

Furthermore, while the discussion of the invention focuses, and is exemplified using human sequences and samples, the invention is equally applicable, through construction or selection of appropriate candidate libraries, to non-human animals, such as laboratory animals, e.g., mice, rats, guinea pigs, rabbits; domesticated livestock, e.g., cows, horses, goats, sheep, chicken, etc.; and companion animals, e.g., dogs, cats, etc.

Methods for obtaining expression data

Numerous methods for obtaining expression data are known, and any one or more of these techniques, singly or in combination, are suitable for determining expression profiles in the context of the present invention. For example, expression patterns can be evaluated by northern analysis, PCR, RT-PCR, Taq Man analysis, FRET detection, monitoring one or more molecular beacon, hybridization to an oligonucleotide array, hybridization to a cDNA array, hybridization to a polynucleotide array, hybridization to a liquid microarray, hybridization to a microelectric array, molecular beacons, cDNA sequencing, clone hybridization, cDNA fragment fingerprinting, serial analysis of gene expression (SAGE), subtractive hybridization, differential display and/or differential screening (*see*, e.g., Lockhart and Winzeler (2000) Nature 405:827-836, and references cited therein).

For example, specific PCR primers are designed to a member(s) of a candidate nucleotide library. cDNA is prepared from subject sample RNA by reverse transcription from a poly-dT oligonucleotide primer, and subjected to PCR. Double stranded cDNA may be prepared using primers suitable for reverse transcription of the PCR product, followed by amplification of the cDNA using in

vitro transcription. The product of in vitro transcription is a sense-RNA corresponding to the original member(s) of the candidate library. PCR product may be also be evaluated in a number of ways known in the art, including real-time assessment using detection of labeled primers, e.g. TaqMan or molecular beacon probes. Technology platforms suitable for analysis of PCR products include the ABI 7700, 5700, or 7000 Sequence Detection Systems (Applied Biosystems, Foster City, CA), the MJ Research Opticon (MJ Research, Waltham, MA), the Roche Light Cycler (Roche Diagnositics, Indianapolis, IN), the Stratagene MX4000 (Stratagene, La Jolla, CA), and the Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA). Alternatively, molecular beacons are used to detect presence of a nucleic acid sequence in an unamplified RNA or cDNA sample, or following amplification of the sequence using any method, e.g. IVT (In Vitro transcription) or NASBA (nucleic acid sequence based amplification). Molecular beacons are designed with sequences complementary to member(s) of a candidate nucleotide library, and are linked to fluorescent labels. Each probe has a different fluorescent label with non-overlapping emission wavelengths. For example, expression of ten genes may be assessed using ten different sequence-specific molecular beacons.

Alternatively, or in addition, molecular beacons are used to assess expression of multiple nucleotide sequences at once. Molecular beacons with sequence complimentary to the members of a diagnostic nucleotide set are designed and linked to fluorescent labels. Each fluorescent label used must have a non-overlapping emission wavelength. For example, 10 nucleotide sequences can be assessed by hybridizing 10 sequence specific molecular beacons (each labeled with a different fluorescent molecule) to an amplified or un-amplified RNA or cDNA sample. Such an assay bypasses the need for sample labeling procedures.

Alternatively, or in addition bead arrays can be used to assess expression of multiple sequences at once (See, e.g, LabMAP 100, Luminex Corp, Austin, Texas). Alternatively, or in addition electric arrays are used to assess expression of multiple sequences, as exemplified by the e-Sensor technology of Motorola (Chicago, Ill.) or Nanochip technology of Nanogen (San Diego, CA.)

Of course, the particular method elected will be dependent on such factors as quantity of RNA recovered, practitioner preference, available reagents and equipment, detectors, and the like. Typically, however, the elected method(s) will be appropriate for processing the number of samples and probes of interest. Methods for high-throughput expression analysis are discussed below.

Alternatively, expression at the level of protein products of gene expression is performed. For example, protein expression, in a sample of leukocytes, can be evaluated by one or more method selected from among: western analysis, two-dimensional gel analysis, chromatographic separation, mass spectrometric detection, protein-fusion reporter constructs, colorimetric assays, binding to a protein array and characterization of polysomal mRNA. One particularly favorable approach involves binding of labeled protein expression products to an array of antibodies specific for members of the candidate library. Methods for producing and evaluating antibodies are widespread in the art, *see*, e.g., Coligan, *supra*; and Harlow and Lane (1989) Antibodies: A Laboratory Manual, Cold Spring Harbor Press, NY ("Harlow and Lane"). Additional details regarding a variety of immunological and immunoassay procedures adaptable to the present invention by selection of antibody reagents specific for the products of candidate nucleotide sequences can be found in, e.g., Stites and Terr (eds.)(1991)

Basic and Clinical Immunology, 7th ed., and Paul, *supra*. Another approach uses systems for performing desorption spectrometry. Commercially available systems, e.g., from Ciphergen Biosystems, Inc. (Fremont, CA) are particularly well suited to quantitative analysis of protein expression. Indeed, Protein Chip® arrays (*see*, e.g., the website, ciphergen.com) used in desorption spectrometry approaches provide arrays for detection of protein expression. Alternatively, affinity reagents, (e.g., antibodies, small molecules, etc.) are developed that recognize epitopes of the protein product. Affinity assays are used in protein array assays, e.g. to detect the presence or absence of particular proteins. Alternatively, affinity reagents are used to detect expression using the methods described above. In the case of a protein that is expressed on the cell surface of leukocytes, labeled affinity reagents are bound to populations of leukocytes, and leukocytes expressing the protein are identified and counted using fluorescent activated cell sorting (FACS).

It is appreciated that the methods of expression evaluation discussed herein, although discussed in the context of discovery of diagnostic nucleotide sets, are also applicable for expression evaluation when using diagnostic nucleotide sets for, e.g. diagnosis of diseases, as further discussed below.

High Throughput Expression Assays

A number of suitable high throughput formats exist for evaluating gene expression. Typically, the term high throughput refers to a format that performs at least about 100 assays, or at least about 500 assays, or at least about 1000 assays, or at least about 5000 assays, or at least about 10,000 assays, or more per day. When enumerating assays, either the number of samples or the number of candidate nucleotide sequences evaluated can be considered. For example, a northern analysis of, e.g., about 100 samples performed in a gridded array, e.g., a dot blot, using a single probe corresponding to a candidate nucleotide sequence can be considered a high throughput assay. More typically, however, such an assay is performed as a series of duplicate blots, each evaluated with a distinct probe corresponding to a different member of the candidate library. Alternatively, methods that simultaneously evaluate expression of about 100 or more candidate nucleotide sequences in one or more samples, or in multiple samples, are considered high throughput.

Numerous technological platforms for performing high throughput expression analysis are known. Generally, such methods involve a logical or physical array of either the subject samples, or the candidate library, or both. Common array formats include both liquid and solid phase arrays. For example, assays employing liquid phase arrays, e.g., for hybridization of nucleic acids, binding of antibodies or other receptors to ligand, etc., can be performed in multiwell, or microtiter, plates. Microtiter plates with 96, 384 or 1536 wells are widely available, and even higher numbers of wells, e.g, 3456 and 9600 can be used. In general, the choice of microtiter plates is determined by the methods and equipment, e.g., robotic handling and loading systems, used for sample preparation and analysis. Exemplary systems include, e.g., the ORCA™ system from Beckman-Coulter, Inc. (Fullerton, CA) and the Zymate systems from Zymark Corporation (Hopkinton, MA).

Alternatively, a variety of solid phase arrays can favorably be employed in to determine expression patterns in the context of the invention. Exemplary formats include membrane or filter arrays (e.g, nitrocellulose, nylon), pin arrays, and bead arrays (e.g., in a liquid "slurry"). Typically,

probes corresponding to nucleic acid or protein reagents that specifically interact with (e.g., hybridize to or bind to) an expression product corresponding to a member of the candidate library are immobilized, for example by direct or indirect cross-linking, to the solid support. Essentially any solid support capable of withstanding the reagents and conditions necessary for performing the particular expression assay can be utilized. For example, functionalized glass, silicon, silicon dioxide, modified silicon, any of a variety of polymers, such as (poly)tetrafluoroethylene, (poly)vinylidenedifluoride, polystyrene, polycarbonate, or combinations thereof can all serve as the substrate for a solid phase array.

In a preferred embodiment, the array is a "chip" composed, e.g., of one of the above-specified materials. Polynucleotide probes, e.g., RNA or DNA, such as cDNA, synthetic oligonucleotides, and the like, or binding proteins such as antibodies, that specifically interact with expression products of individual components of the candidate library are affixed to the chip in a logically ordered manner, i.e., in an array. In addition, any molecule with a specific affinity for either the sense or anti-sense sequence of the marker nucleotide sequence (depending on the design of the sample labeling), can be fixed to the array surface without loss of specific affinity for the marker and can be obtained and produced for array production, for example, proteins that specifically recognize the specific nucleic acid sequence of the marker, ribozymes, peptide nucleic acids (PNA), or other chemicals or molecules with specific affinity.

Detailed discussion of methods for linking nucleic acids and proteins to a chip substrate, are found in, e.g., US Patent No. 5,143,854 "Large Scale Photolithographic Solid Phase Synthesis Of Polypeptides And Receptor Binding Screening Thereof" to Pirrung et al., issued, September 1, 1992; US Patent No. 5,837,832 "Arrays Of Nucleic Acid Probes On Biological Chips" to Chee et al., issued November 17, 1998; US Patent No. 6,087,112 "Arrays With Modified Oligonucleotide And Polynucleotide Compositions" to Dale, issued July 11, 2000; US Patent No. 5,215,882 "Method Of Immobilizing Nucleic Acid On A Solid Substrate For Use In Nucleic Acid Hybridization Assays" to Bahl et al., issued June 1, 1993; US Patent No. 5,707,807 "Molecular Indexing For Expressed Gene Analysis" to Kato, issued January 13, 1998; US Patent No. 5,807,522 "Methods For Fabricating Microarrays Of Biological Samples" to Brown et al., issued September 15, 1998; US Patent No. 5,958,342 "Jet Droplet Device" to Gamble et al., issued Sept. 28, 1999; US Patent 5,994,076 "Methods Of Assaying Differential Expression" to Chenchik et al., issued Nov. 30, 1999; US Patent No. 6,004,755 "Quantitative Microarray Hybridization Assays" to Wang, issued Dec. 21, 1999; US Patent No. 6,048,695 "Chemically Modified Nucleic Acids And Method For Coupling Nucleic Acids To Solid Support" to Bradley et al., issued April 11, 2000; US Patent No. 6,060,240 "Methods For Measuring Relative Amounts Of Nucleic Acids In A Complex Mixture And Retrieval Of Specific Sequences Therefrom" to Kamb et al., issued May 9, 2000; US Patent No. 6,090,556 "Method For Quantitatively Determining The Expression Of A Gene" to Kato, issued July 18, 2000; and US Patent 6,040,138 "Expression Monitoring By Hybridization To High Density Oligonucleotide Arrays" to Lockhart et al., issued March 21, 2000 each of which are hereby incorporated by reference in their entirety.

For example, cDNA inserts corresponding to candidate nucleotide sequences, in a standard TA cloning vector are amplified by a polymerase chain reaction for approximately 30-40 cycles. The amplified PCR products are then arrayed onto a glass support by any of a variety of well-known techniques, e.g., the VSLIPS™ technology described in US Patent No. 5,143,854. RNA, or cDNA corresponding to RNA, isolated from a subject sample of leukocytes is labeled, e.g., with a fluorescent tag, and a solution containing the RNA (or cDNA) is incubated under conditions favorable for hybridization, with the "probe" chip. Following incubation, and washing to eliminate non-specific hybridization, the labeled nucleic acid bound to the chip is detected qualitatively or quantitatively, and the resulting expression profile for the corresponding candidate nucleotide sequences is recorded. It is appreciated that the probe used for diagnostic purposes may be identical to the probe used during diagnostic nucleotide sequence discovery and validation. Alternatively, the probe sequence may be different than the sequence used in diagnostic nucleotide sequence discovery and validation. Multiple cDNAs from a nucleotide sequence that are non-overlapping or partially overlapping may also be used.

In another approach, oligonucleotides corresponding to members of a candidate nucleotide library are synthesized and spotted onto an array. Alternatively, oligonucleotides are synthesized onto the array using methods known in the art, e.g. Hughes, et al. *supra*. The oligonucleotide is designed to be complementary to any portion of the candidate nucleotide sequence. In addition, in the context of expression analysis for, e.g. diagnostic use of diagnostic nucleotide sets, an oligonucleotide can be designed to exhibit particular hybridization characteristics, or to exhibit a particular specificity and/or sensitivity, as further described below.

Hybridization signal may be amplified using methods known in the art, and as described herein, for example use of the Clontech kit (Glass Fluorescent Labeling Kit), Stratagene kit (Fairplay Microarray Labeling Kit), the Micromax kit (New England Nuclear, Inc.), the Genisphere kit (3DNA Submicro), linear amplification, e.g. as described in U.S. Patent No. 6,132,997 or described in Hughes, TR, et al., Nature Biotechnology, 19:343-347 (2001) and/or Westin et al. Nat Biotech. 18:199-204. In some cases, amplification techniques do not increase signal intensity, but allow assays to be done with small amounts of RNA.

Alternatively, fluorescently labeled cDNA are hybridized directly to the microarray using methods known in the art. For example, labeled cDNA are generated by reverse transcription using Cy3- and Cy5-conjugated deoxynucleotides, and the reaction products purified using standard methods. It is appreciated that the methods for signal amplification of expression data useful for identifying diagnostic nucleotide sets are also useful for amplification of expression data for diagnostic purposes.

Microarray expression may be detected by scanning the microarray with a variety of laser or CCD-based scanners, and extracting features with numerous software packages, for example, Imagene (Biodiscovery), Feature Extraction Software (Agilent), Scanalyze (Eisen, M. 1999. SCANALYZE User Manual; Stanford Univ., Stanford, CA. Ver 2.32.), GenePix (Axon Instruments).

In another approach, hybridization to microelectric arrays is performed, e.g. as described in Umek et al (2001) J Mol Diagn. 3:74-84. An affinity probe, e.g. DNA, is deposited on a metal surface. The metal surface underlying each probe is connected to a metal wire and electrical signal detection system. Unlabelled RNA or cDNA is hybridized to the array, or alternatively, RNA or cDNA sample

is amplified before hybridization, e.g. by PCR. Specific hybridization of sample RNA or cDNA results in generation of an electrical signal, which is transmitted to a detector. See Westin (2000) Nat Biotech. 18:199-204 (describing anchored multiplex amplification of a microelectronic chip array); Edman (1997) NAR 25:4907-14; Vignali (2000) J Immunol Methods 243:243-55.

In another approach, a microfluidics chip is used for RNA sample preparation and analysis. This approach increases efficiency because sample preparation and analysis are streamlined. Briefly, microfluidics may be used to sort specific leukocyte sub-populations prior to RNA preparation and analysis. Microfluidics chips are also useful for, e.g., RNA preparation, and reactions involving RNA (reverse transcription, RT-PCR). Briefly, a small volume of whole, anti-coagulated blood is loaded onto a microfluidics chip, for example chips available from Caliper (Mountain View, CA) or Nanogen (San Diego, CA.) A microfluidics chip may contain channels and reservoirs in which cells are moved and reactions are performed. Mechanical, electrical, magnetic, gravitational, centrifugal or other forces are used to move the cells and to expose them to reagents. For example, cells of whole blood are moved into a chamber containing hypotonic saline, which results in selective lysis of red blood cells after a 20-minute incubation. Next, the remaining cells (leukocytes) are moved into a wash chamber and finally, moved into a chamber containing a lysis buffer such as guanidine isothyocyanate. The leukocyte cell lysate is further processed for RNA isolation in the chip, or is then removed for further processing, for example, RNA extraction by standard methods. Alternatively, the microfluidics chip is a circular disk containing ficoll or another density reagent. The blood sample is injected into the center of the disc, the disc is rotated at a speed that generates a centrifugal force appropriate for density gradient separation of mononuclear cells, and the separated mononuclear cells are then harvested for further analysis or processing.

It is understood that the methods of expression evaluation, above, although discussed in the context of discovery of diagnostic nucleotide sets, are also applicable for expression evaluation when using diagnostic nucleotide sets for, e.g. diagnosis of diseases, as further discussed below.

Evaluation of expression patterns

Expression patterns can be evaluated by qualitative and/or quantitative measures. Certain of the above described techniques for evaluating gene expression (as RNA or protein products) yield data that are predominantly qualitative in nature. That is, the methods detect differences in expression that classify expression into distinct modes without providing significant information regarding quantitative aspects of expression. For example, a technique can be described as a qualitative technique if it detects the presence or absence of expression of a candidate nucleotide sequence, i.e., an on/off pattern of expression. Alternatively, a qualitative technique measures the presence (and/or absence) of different alleles, or variants, of a gene product.

In contrast, some methods provide data that characterize expression in a quantitative manner. That is, the methods relate expression on a numerical scale, e.g., a scale of 0-5, a scale of 1-10, a scale of + - +++, from grade 1 to grade 5, a grade from a to z, or the like. It will be understood that the numerical, and symbolic examples provided are arbitrary, and that any graduated scale (or any symbolic representation of a graduated scale) can be employed in the context of the present invention

to describe quantitative differences in nucleotide sequence expression. Typically, such methods yield information corresponding to a relative increase or decrease in expression.

Any method that yields either quantitative or qualitative expression data is suitable for evaluating expression of candidate nucleotide sequence in a subject sample of leukocytes. In some cases, e.g., when multiple methods are employed to determine expression patterns for a plurality of candidate nucleotide sequences, the recovered data, e.g., the expression profile, for the nucleotide sequences is a combination of quantitative and qualitative data.

In some applications, expression of the plurality of candidate nucleotide sequences is evaluated sequentially. This is typically the case for methods that can be characterized as low- to moderate-throughput. In contrast, as the throughput of the elected assay increases, expression for the plurality of candidate nucleotide sequences in a sample or multiple samples of leukocytes, is assayed simultaneously. Again, the methods (and throughput) are largely determined by the individual practitioner, although, typically, it is preferable to employ methods that permit rapid, e.g. automated or partially automated, preparation and detection, on a scale that is time-efficient and cost-effective.

It is understood that the preceding discussion, while directed at the assessment of expression of the members of candidate libraries, is also applies to the assessment of the expression of members of diagnostic nucleotide sets, as further discussed below.

Genotyping

In addition to, or in conjunction with the correlation of expression profiles and clinical data, it is often desirable to correlate expression patterns with the subject's genotype at one or more genetic loci or to correlate both expression profiles and genetic loci data with clinical data. The selected loci can be, for example, chromosomal loci corresponding to one or more member of the candidate library, polymorphic alleles for marker loci, or alternative disease related loci (not contributing to the candidate library) known to be, or putatively associated with, a disease (or disease criterion). Indeed, it will be appreciated, that where a (polymorphic) allele at a locus is linked to a disease (or to a predisposition to a disease), the presence of the allele can itself be a disease criterion.

Numerous well known methods exist for evaluating the genotype of an individual, including southern analysis, restriction fragment length polymorphism (RFLP) analysis, polymerase chain reaction (PCR), amplification length polymorphism (AFLP) analysis, single stranded conformation polymorphism (SSCP) analysis, single nucleotide polymorphism (SNP) analysis (e.g., via PCR, Taqman or molecular beacons), among many other useful methods. Many such procedures are readily adaptable to high throughput and/or automated (or semi-automated) sample preparation and analysis methods. Most, can be performed on nucleic acid samples recovered via simple procedures from the same sample of leukocytes as yielded the material for expression profiling. Exemplary techniques are described in, e.g., Sambrook, and Ausubel, *supra*.

Identification of the diagnostic oligonucleotides and oligonucleotide sets of the invention

Identification of diagnostic nucleotides and nucleotide sets and disease specific target nucleotide sequence proceeds by correlating the leukocyte expression profiles with data regarding the subject's health status to produce a data set designated a "molecular signature." Examples of data

regarding a patient's health status, also termed "disease criteria(ion)", is described below and in the Section titled "selected diseases," below. Methods useful for correlation analysis are further described elsewhere in the specification.

Generally, relevant data regarding the subject's health status includes retrospective or prospective health data, e.g., in the form of the subject's medical history, as provided by the subject, physician or third party, such as, medical diagnoses, laboratory test results, diagnostic test results, clinical events, or medication lists, as further described below. Such data may include information regarding a patient's response to treatment and/or a particular medication and data regarding the presence of previously characterized "risk factors." For example, cigarette smoking and obesity are previously identified risk factors for heart disease. Further examples of health status information, including diseases and disease criteria, is described in the section titled Selected diseases, below.

Typically, the data describes prior events and evaluations (i.e., retrospective data). However, it is envisioned that data collected subsequent to the sampling (i.e., prospective data) can also be correlated with the expression profile. The tissue sampled, e.g., peripheral blood, bronchial lavage, etc., can be obtained at one or more multiple time points and subject data is considered retrospective or prospective with respect to the time of sample procurement.

Data collected at multiple time points, called "longitudinal data", is often useful, and thus, the invention encompasses the analysis of patient data collected from the same patient at different time points. Analysis of paired samples, such as samples from a patient at different times, allows identification of differences that are specifically related to the disease state since the genetic variability specific to the patient is controlled for by the comparison. Additionally, other variables that exist between patients may be controlled for in this way, for example, the presence or absence of inflammatory diseases (e.g., rheumatoid arthritis) the use of medications that may effect leukocyte gene expression, the presence or absence of co-morbid conditions, etc. Methods for analysis of paired samples are further described below. Moreover, the analysis of a pattern of expression profiles (generated by collecting multiple expression profiles) provides information relating to changes in expression level over time, and may permit the determination of a rate of change, a trajectory, or an expression curve. Two longitudinal samples may provide information on the change in expression of a gene over time, while three longitudinal samples may be necessary to determine the "trajectory" of expression of a gene. Such information may be relevant to the diagnosis of a disease. For example, the expression of a gene may vary from individual to individual, but a clinical event, for example, a heart attack, may cause the level of expression to double in each patient. In this example, clinically interesting information is gleaned from the change in expression level, as opposed to the absolute level of expression in each individual.

When a single patient sample is obtained, it may still be desirable to compare the expression profile of that sample to some reference expression profile. In this case, one can determine the change of expression between the patient's sample and a reference expression profile that is appropriate for that patient and the medical condition in question. For example, a reference expression profile can be determined for all patients without the disease criterion in question who have similar characteristics, such as age, sex, race, diagnoses etc.

Generally, small sample sizes of 10-40 samples from 10-20 individuals are used to identify a diagnostic nucleotide set. Larger sample sizes are generally necessary to validate the diagnostic nucleotide set for use in large and varied patient populations, as further described below. For example, extension of gene expression correlations to varied ethnic groups, demographic groups, nations, peoples or races may require expression correlation experiments on the population of interest.

Expression Reference Standards

Expression profiles derived from a patient (i.e., subjects diagnosed with, or exhibiting symptoms of, or exhibiting a disease criterion, or under a doctor's care for a disease) sample are compared to a control or standard expression RNA to facilitate comparison of expression profiles (e.g. of a set of candidate nucleotide sequences) from a group of patients relative to each other (i.e., from one patient in the group to other patients in the group, or to patients in another group).

The reference RNA used should have desirable features of low cost and simplicity of production on a large scale. Additionally, the reference RNA should contain measurable amounts of as many of the genes of the candidate library as possible.

For example, in one approach to identifying diagnostic nucleotide sets, expression profiles derived from patient samples are compared to a expression reference "standard." Standard expression reference can be, for example, RNA derived from resting cultured leukocytes or commercially available reference RNA, such as Universal reference RNA from Stratagene. *See* Nature, V406, 8-17-00, p. 747-752. Use of an expression reference standard is particularly useful when the expression of large numbers of nucleotide sequences is assayed, e.g. in an array, and in certain other applications, e.g. qualitative PCR, RT-PCR, etc., where it is desirable to compare a sample profile to a standard profile, and/or when large numbers of expression profiles, e.g. a patient population, are to be compared. Generally, an expression reference standard should be available in large quantities, should be a good substrate for amplification and labeling reactions, and should be capable of detecting a large percentage of candidate nucleic acids using suitable expression profiling technology.

Alternatively, or in addition, the expression profile derived from a patient sample is compared with the expression of an internal reference control gene, for example, β-actin or CD4. The relative expression of the profiled genes and the internal reference control gene (from the same individual) is obtained. An internal reference control may also be used with a reference RNA. For example, an expression profile for "gene 1" and the gene encoding CD4 can be determined in a patient sample and in a reference RNA. The expression of each gene can be expressed as the "relative" ratio of expression the gene in the patient sample compared with expression of the gene in the reference RNA. The expression ratio (sample/reference) for gene 1 may be divided by the expression ration for CD4 (sample/reference) and thus the relative expression of gene 1 to CD4 is obtained.

The invention also provides a buffy coat control RNA useful for expression profiling, and a method of using control RNA produced from a population of buffy coat cells, the white blood cell layer derived from the centrifugation of whole blood. Buffy coat contains all white blood cells, including granulocytes, mononuclear cells and platelets. The invention also provides a method of preparing control RNA from buffy coat cells for use in expression profile analysis of leukocytes. Buffy coat fractions are obtained, e.g. from a blood bank or directly from individuals, preferably from a large

number of individuals such that bias from individual samples is avoided and so that the RNA sample represents an average expression of a healthy population. Buffy coat fractions from about 50 or about 100, or more individuals are preferred. 10 ml buffy coat from each individual is used. Buffy coat samples are treated with an erthythrocyte lysis buffer, so that erthythrocytes are selectively removed. The leukocytes of the buffy coat layer are collected by centrifugation. Alternatively, the buffy cell sample can be further enriched for a particular leukocyte sub-populations, e.g. mononuclear cells, T-lymphocytes, etc. To enrich for mononuclear cells, the buffy cell pellet, above, is diluted in PBS (phosphate buffered saline) and loaded onto a non-polystyrene tube containing a polysucrose and sodium diatrizoate solution adjusted to a density of 1.077+/-0.001 g/ml. To enrich for T-lymphocytes, 45 ml of whole blood is treated with RosetteSep (Stem Cell Technologies), and incubated at room temperature for 20 minutes. The mixture is diluted with an equal volume of PBS plus 2% FBS and mixed by inversion. 30 ml of diluted mixture is layered on top of 15 ml DML medium (Stem Cell Technologies). The tube is centrifuged at 1200 x g, and the enriched cell layer at the plasma : medium interface is removed, washed with PBS + 2% FBS, and cells collected by centrifugation at 1200 x g. The cell pellet is treated with 5 ml of erythrocyte lysis buffer (EL buffer, Qiagen) for 10 minutes on ice, and enriched T-lymphoctes are collected by centrifugation.

In addition or alternatively, the buffy cells (whole buffy coat or sub-population, e.g. mononuclear fraction) can be cultured *in vitro* and subjected to stimulation with cytokines or activating chemicals such as phorbol esters or ionomycin. Such stimuli may increase expression of nucleotide sequences that are expressed in activated immune cells and might be of interest for leukocyte expression profiling experiments.

Following sub-population selection and/or further treatment, e.g. stimulation as described above, RNA is prepared using standard methods. For example, cells are pelleted and lysed with a phenol/guanidinium thiocyanate and RNA is prepared. RNA can also be isolated using a silica gel-based purification column or the column method can be used on RNA isolated by the phenol/guanidinium thiocyanate method. RNA from individual buffy coat samples can be pooled during this process, so that the resulting reference RNA represents the RNA of many individuals and individual bias is minimized or eliminated. In addition, a new batch of buffy coat reference RNA can be directly compared to the last batch to ensure similar expression pattern from one batch to another, using methods of collecting and comparing expression profiles described above/below. One or more expression reference controls are used in an experiment. For example, RNA derived from one or more of the following sources can be used as controls for an experiment: stimulated or unstimulated whole buffy coat, stimulated or unstimulated peripheral mononuclear cells, or stimulated or unstimulated T-lymphocytes.

Alternatively, the expression reference standard can be derived from any subject or class of subjects including healthy subjects or subjects diagnosed with the same or a different disease or disease criterion. Expression profiles from subjects in two or more distinct classes are compared to determine which subset of nucleotide sequences in the candidate library can best distinguish between the subject classes, as further discussed below. It will be appreciated that in the present context, the term "distinct classes" is relevant to at least one distinguishable criterion relevant to a disease of interest, a "disease

criterion." The classes can, of course, demonstrate significant overlap (or identity) with respect to other disease criteria, or with respect to disease diagnoses, prognoses, or the like. The mode of discovery involves, e.g., comparing the molecular signature of different subject classes to each other (such as patient to control, patients with a first diagnosis to patients with a second diagnosis, etc.) or by comparing the molecular signatures of a single individual taken at different time points. The invention can be applied to a broad range of diseases, disease criteria, conditions and other clinical and/or epidemiological questions, as further discussed above/below.

It is appreciated that while the present discussion pertains to the use of expression reference controls while identifying diagnostic nucleotide sets, expression reference controls are also useful during use of diagnostic nucleotide sets, e.g. use of a diagnostic nucleotide set for diagnosis of a disease, as further described below.

Analysis of expression profiles

In order to facilitate ready access, e.g., for comparison, review, recovery, and/or modification, the molecular signatures/expression profiles are typically recorded in a database. Most typically, the database is a relational database accessible by a computational device, although other formats, e.g., manually accessible indexed files of expression profiles as photographs, analogue or digital imaging readouts, spreadsheets, etc. can be used. Further details regarding preferred embodiments are provided below. Regardless of whether the expression patterns initially recorded are analog or digital in nature and/or whether they represent quantitative or qualitative differences in expression, the expression patterns, expression profiles (collective expression patterns), and molecular signatures (correlated expression patterns) are stored digitally and accessed via a database. Typically, the database is compiled and maintained at a central facility, with access being available locally and/or remotely.

As additional samples are obtained, and their expression profiles determined and correlated with relevant subject data, the ensuing molecular signatures are likewise recorded in the database. However, rather than each subsequent addition being added in an essentially passive manner in which the data from one sample has little relation to data from a second (prior or subsequent) sample, the algorithms optionally additionally query additional samples against the existing database to further refine the association between a molecular signature and disease criterion. Furthermore, the data set comprising the one (or more) molecular signatures is optionally queried against an expanding set of additional or other disease criteria. The use of the database in integrated systems and web embodiments is further described below.

Analysis of expression profile data from arrays

Expression data is analyzed using methods well known in the art, including the software packages Imagene (Biodiscovery, Marina del Rey, CA), Feature Extraction Software (Agilent, Palo Alto, CA), and Scanalyze (Stanford University). In the discussion that follows, a "feature" refers to an individual spot of DNA on an array. Each gene may be represented by more than one feature. For example, hybridized microarrays are scanned and analyzed on an Axon Instruments scanner using GenePix 3.0 software (Axon Instruments, Union City, CA). The data extracted by GenePix is used for all downstream quality control and expression evaluation. The data is derived as follows. The data for all features flagged as "not found" by the software is removed from the dataset for individual

hybridizations. The "not found" flag by GenePix indicates that the software was unable to discriminate the feature from the background. Each feature is examined to determine the value of its signal. The median pixel intensity of the background (B_n) is subtracted from the median pixel intensity of the feature (F_n) to produce the background-subtracted signal (hereinafter, "BGSS"). The BGSS is divided by the standard deviation of the background pixels to provide the signal-to-noise ratio (hereinafter, "S/N"). Features with a S/N of three or greater in both the Cy3 channel (corresponding to the sample RNA) and Cy5 channel (corresponding to the reference RNA) are used for further analysis (hereinafter denoted "useable features"). Alternatively, different S/Ns are used for selecting expression data for an analysis. For example, only expression data with signal to noise ratios > 3 might be used in an analysis. Alternatively, features with S/N values < 3 may be flagged as such and included in the analysis. Such flagged data sets include more values and may allow one to discover expression markers that would be missed otherwise. However, such data sets may have a higher variablilty than filtered data, which may decrease significance of findings or performance of correlation statistics.

For each usable feature (*i*), the expression level (*e*) is expressed as the logarithm of the ratio (R) of the Background Subtracted Signal (hereinafter "BGSS") for the Cy3 (sample RNA) channel divided by the BGSS for the Cy5 channel (reference RNA). This "log ratio" value is used for comparison to other experiments.

$$R_i = \frac{BGSS_{sample}}{BGSS_{reference}} \tag{0.1}$$

$$e_i = \log r_i \tag{0.2}$$

Variation in signal across hybridizations may be caused by a number of factors affecting hybridization, DNA spotting, wash conditions, and labeling efficiency.

A single reference RNA may be used with all of the experimental RNAs, permitting multiple comparisons in addition to individual comparisons. By comparing sample RNAs to the same reference, the gene expression levels from each sample are compared across arrays, permitting the use of a consistent denominator for our experimental ratios. Alternative methods of analyzing the data may involve 1) using the sample channel without normalization by the reference channel, 2) using an intensity-dependent normalization based on the reference which provides a greater correction when the signal in the reference channel is large, 3) using the data without background subtraction or subtracting an empirically derived function of the background intensity rather than the background itself.

Scaling

The data may be scaled (normalized) to control for labeling and hybridization variability within the experiment, using methods known in the art. Scaling is desirable because it facilitates the comparison of data between different experiments, patients, etc. Generally the BGSS are scaled to a factor such as the median, the mean, the trimmed mean, and percentile. Additional methods of scaling include: to scale between 0 and 1, to subtract the mean, or to subtract the median.

Scaling is also performed by comparison to expression patterns obtained using a common reference RNA, as described in greater detail above. As with other scaling methods, the reference RNA facilitates multiple comparisons of the expression data, e.g., between patients, between samples, etc. Use of a reference RNA provides a consistent denominator for experimental ratios.

In addition to the use of a reference RNA, individual expression levels may be adjusted to correct for differences in labeling efficiency between different hybridization experiments, allowing direct comparison between experiments with different overall signal intensities, for example. A scaling factor (a) may be used to adjust individual expression levels as follows. The median of the scaling factor (a), for example, BGSS, is determined for the set of all features with a S/N greater than three. Next, the $BGSS_i$ (the BGSS for each feature "i") is divided by the median for all features (a), generating a scaled ratio. The scaled ration is used to determine the expression value for the feature (e_i), or the log ratio.

$$S_i = \frac{BGSS_i}{a} \tag{0.3}$$

$$e_i = \log\left(\frac{Cy3S_i}{Cy5S_i}\right) \tag{0.4}$$

In addition, or alternatively, control features are used to normalize the data for labeling and hybridization variability within the experiment. Control feature may be cDNA for genes from the plant, *Arabidopsis thaliana*, that are included when spotting the mini-array. Equal amounts of RNA complementary to control cDNAs are added to each of the samples before they were labeled. Using the signal from these control genes, a normalization constant (L) is determined according to the following formula:

$$L_j = \frac{\dfrac{\sum_{i=1}^{N} BGSS_{j,i}}{N}}{\dfrac{\sum_{j=1}^{K} \dfrac{\sum_{i=1}^{N} BGSS_{j,i}}{N}}{K}},$$

where $BGSS_i$ is the signal for a specific feature, N is the number of *A. thaliana* control features, K is the number of hybridizations, and L_j is the normalization constant for each individual hybridization.

Using the formula above, the mean for all control features of a particular hybridization and dye (e.g., Cy3) is calculated. The control feature means for all Cy3 hybridizations are averaged, and the control feature mean in one hybridization divided by the average of all hybridizations to generate a normalization constant for that particular Cy3 hybridization (L_j), which is used as a in equation (0.3). The same normalization steps may be performed for Cy3 and Cy5 values.

An alternative scaling method can also be used. The log of the ratio of Green/Red is determined for all features. The median log ratio value for all features is determined. The feature values are then scaled using the following formula: Log_Scaled_Feature_Ratio = Log_Feature_Ratio – Median_Log_Ratio.

Many additional methods for normalization exist and can be applied to the data. In one method, the average ratio of Cy3 BGSS / Cy5 BGSS is determined for all features on an array. This ratio is then scaled to some arbitrary number, such as 1 or some other number. The ratio for each probe is then multiplied by the scaling factor required to bring the average ratio to the chosen level. This is performed for each array in an analysis. Alternatively, the ratios are normalized to the average ratio across all arrays in an analysis. Other methods of normalization include forcing the distribution of signal strengths of the various arrays into greater agreement by transforming them to match certain points (quartiles, or deciles, etc.) in a standard distribution, or in the most extreme case using the rank of the signal of each oligonucleotide relative to the other oligonucleotides on the array

If multiple features are used per gene sequence or oligonucleotide, these repeats can be used to derive an average expression value for each gene. If some of the replicate features are of poor qualitay and don't meet requirements for analysis, the remaining features can be used to represent the gene or gene sequence.

Correlation analysis

Correlation analysis is performed to determine which array probes have expression behavior that best distinguishes or serves as markers for relevant groups of samples representing a particular clinical condition. Correlation analysis, or comparison among samples representing different disease criteria (e.g., clinical conditions), is performed using standard statistical methods. Numerous algorithms are useful for correlation analysis of expression data, and the selection of algorithms depends in part on the data analysis to be performed. For example, algorithms can be used to identify the single most informative gene with expression behavior that reliably classifies samples, or to identify all the genes useful to classify samples. Alternatively, algorithms can be applied that determine which set of 2 or more genes have collective expression behavior that accurately classifies samples. The use of multiple expression markers for diagnostics may overcome the variability in expression of a gene between individuals, or overcome the variability intrinsic to the assay. Multiple expression markers may include redundant markers (surrogates), in that two or more genes or probes may provide the same information with respect to diagnosis. This may occur, for example, when two or more genes or gene probes are coordinately expressed. For diagnostic application, it may be appropriate to utilize a gene and one or more of its surrogates in the assay. This redundancy may overcome failures (technical or biological) of a single marker to distinguish samples. Alternatively, one or more surrogates may have properties that make them more suitable for assay development, such as a higher baseline level of expression, better cell specificity, a higher fold change between sample groups or more specific sequence for the design of PCR primers or complimentary probes. It will be appreciated that while the discussion above pertains to the analysis of RNA expression profiles the discussion is equally applicable to the analysis of profiles of proteins or other molecular markers.

Prior to analysis, expression profile data may be formatted or prepared for analysis using methods known in the art. For example, often the log ratio of scaled expression data for every array probe is calculated using the following formula:

log (Cy 3 BGSS/ Cy5 BGSS), where Cy 3 signal corresponds to the expression of the gene in the clinical sample, and Cy5 signal corresponds to expression of the gene in the reference RNA.

Data may be further filtered depending on the specific analysis to be done as noted below. For example, filtering may be aimed at selecting only samples with expression above a certain level, or probes with variability above a certain level between sample sets.

The following non-limiting discussion consider several statistical methods known in the art. Briefly, the t-test and ANOVA are used to identify single genes with expression differences between or among populations, respectively. Multivariate methods are used to identify a set of two or more genes for which expression discriminates between two disease states more specifically than expression of any single gene.

t-test

The simplest measure of a difference between two groups is the Student's t test. See, e.g., Welsh et al. (2001) Proc Natl Acad Sci USA 98:1176-81 (demonstrating the use of an unpaired Student's t-test for the discovery of differential gene expression in ovarian cancer samples and control tissue samples). The t- test assumes equal variance and normally distributed data. This test identifies the probability that there is a difference in expression of a single gene between two groups of samples. The number of samples within each group that is required to achieve statistical significance is dependent upon the variation among the samples within each group. The standard formula for a t-test is:

$$t(e_i) = \frac{\bar{e}_{i,c} - \bar{e}_{i,t}}{\sqrt{(s_{i,c}^2/n_c) + (s_{i,t}^2/n_t)}}, \qquad (0.5)$$

where $\bar{e}_i$ is the difference between the mean expression level of gene i in groups c and t, $s_{i,c}$ is the variance of gene x in group c and $s_{i,t}$ is the variance of gene x in group t. n_c and n_t are the numbers of samples in groups c and t.

The combination of the t statistic and the degrees of freedom [min(n_t, n_c)-1] provides a p value, the probability of rejecting the null hypothesis. A p-value of ≤ 0.01, signifying a 99 percent probability the mean expression levels are different between the two groups (a 1% chance that the mean expression levels are in fact not different and that the observed difference occurred by statistical chance), is often considered acceptable.

When performing tests on a large scale, for example, on a large dataset of about 8000 genes, a correction factor must be included to adjust for the number of individual tests being performed. The most common and simplest correction is the Bonferroni correction for multiple tests, which divides the p-value by the number of tests run. Using this test on an 8000 member dataset indicates that a p value

of ≤0.00000125 is required to identify genes that are likely to be truly different between the two test conditions.

Significance analysis for microarrays (SAM)

Significance analysis for microarrays (SAM) (Tusher 2001) is a method through which genes with a correlation between their expression values and the response vector are statistically discovered and assigned a statistical significance. The ratio of false significant to significant genes is the False Discovery Rate (FDR). This means that for each threshold there are a set of genes which are called significant, and the FDR gives a confidence level for this claim. If a gene is called differentially expressed between 2 classes by SAM, with a FDR of 5%, there is a 95% chance that the gene is actually differentially expressed between the classes. SAM takes intoaccount the variability and large number of variables of microarrays. SAM will identiy genes that are most globally differentially expressed between the classes. Thus, important genes for identifying and classifying outlier samples or patients may not be identified by SAM.

Non-Parametric Tests

Wilcoxon's signed ranks method is one example of a non-parametric test and is utilized for paired comparisons. See e.g., Sokal and Rohlf (1987) Introduction to Biostatistics 2nd edition, WH Freeman, New York. At least 6 pairs are necessary to apply this statistic. This test is useful for analysis of paired expression data (for example, a set of patients who have had samples taken before and after administration of a pharmacologic agent). The Fisher Exact Test with a threshold and the Mann-Whitney Test are other non-parametric tests that may be used

ANOVA

Differences in gene expression across multiple related groups may be assessed using an Analysis of Variance (ANOVA), a method well known in the art (Michelson and Schofield, 1996).

Multivariate analysis

Many algorithms suitable for multivariate analysis are known in the art (Katz 1999). Generally, a set of two or more genes for which expression discriminates between two disease states more specifically than expression of any single gene is identified by searching through the possible combinations of genes using a criterion for discrimination, for example the expression of gene X must increase from normal 300 percent, while the expression of genes Y and Z must decrease from normal by 75 percent. Ordinarily, the search starts with a single gene, then adds the next best fit at each step of the search. Alternatively, the search starts with all of the genes and genes that do not aid in the discrimination are eliminated step-wise.

Paired samples

Paired samples, or samples collected at different time-points from the same patient, are often useful, as described above. For example, use of paired samples permits the reduction of variation due to genetic variation among individuals. In addition, the use of paired samples has a statistical significance in that data derived from paired samples can be calculated in a different manner that recognizes the reduced variability. For example, the formula for a t-test for paired samples is:

$$t(e_x) = \frac{\overline{D}_{\bar{e}x}}{\sqrt{\frac{\sum D^2 - (\sum D)^2/b}{b-1}}} \tag{0.5}$$

where D is the difference between each set of paired samples and b is the number of sample pairs. $\overline{D}$ is the mean of the differences between the members of the pairs. In this test, only the differences between the paired samples are considered, then grouped together (as opposed to taking all possible differences between groups, as would be the case with an ordinary t-test). Additional statistical tests useful with paired data, e.g., ANOVA and Wilcoxon's signed rank test, are discussed above.

Diagnostic classification

Once a discriminating set of genes is identified, the diagnostic classifier (a mathematical function that assigns samples to diagnostic categories based on expression data) is applied to unknown sample expression levels.

Methods that can be used for this analysis include the following non-limiting list:

CLEAVER is an algorithm used for classification of useful expression profile data. See Raychaudhuri et al. (2001) Trends Biotechnol 19:189-193. CLEAVER uses positive training samples (e.g., expression profiles from samples known to be derived from a particular patient or sample diagnostic category, disease or disease criteria), negative training samples (e.g., expression profiles from samples known not to be derived from a particular patient or sample diagnostic category, disease or disease criteria) and test samples (e.g., expression profiles obtained from a patient), and determines whether the test sample correlates with the particular disease or disease criteria, or does not correlate with a particular disease or disease criteria. CLEAVER also generates a list of the 20 most predictive genes for classification.

Artificial neural networks (hereinafter, "ANN") can be used to recognize patterns in complex data sets and can discover expression criteria that classify samples into more than 2 groups. The use of artificial neural networks for discovery of gene expression diagnostics for cancers using expression data generated by oligonucleotide expression microarrays is demonstrated by Khan et al. (2001) Nature Med. 7:673-9. Khan found that 96 genes provided 0% error rate in classification of the tumors. The most important of these genes for classification was then determined by measuring the sensitivity of the classification to a change in expression of each gene. Hierarchical clustering using the 96 genes results in correct grouping of the cancers into diagnostic categories.

Golub uses cDNA microarrays and a distinction calculation to identify genes with expression behavior that distinguishes myeloid and lymphoid leukemias. See Golub et al. (1999) Science 286:531-7. Self organizing maps were used for new class discovery. Cross validation was done with a "leave one out" analysis. 50 genes were identified as useful markers. This was reduced to as few as 10 genes with equivalent diagnostic accuracy.

Hierarchical and non-hierarchical clustering methods are also useful for identifying groups of genes that correlate with a subset of clinical samples such as those with and without Lupus. Alizadeh used hierarchical clustering as the primary tool to distinguish different types of diffuse B-cell

lymphomas based on gene expression profile data. See Alizadeh et al. (2000) Nature 403:503-11. Alizadeh used hierarchical clustering as the primary tool to distinguish different types of diffuse B-cell lymphomas based on gene expression profile data. A cDNA array carrying 17856 probes was used for these experiments, 96 samples were assessed on 128 arrays, and a set of 380 genes was identified as being useful for sample classification.

Perou demonstrates the use of hierarchical clustering for the molecular classification of breast tumor samples based on expression profile data. See Perou el al. (2000) Nature 406:747-52. In this work, a cDNA array carrying 8102 gene probes was used. 1753 of these genes were found to have high variation between breast tumors and were used for the analysis.

Hastie describes the use of gene shaving for discovery of expression markers. Hastie et al. (2000) Genome Biol. 1(2):RESEARCH 0003.1-0003.21. The gene shaving algorithm identifies sets of genes with similar or coherent expression patterns, but large variation across conditions (RNA samples, sample classes, patient classes). In this manner, genes with a tight expression pattern within a diagnostic group, but also with high variability across the diagnoses are grouped together. The algorithm takes advantage of both characteristics in one grouping step. For example, gene shaving can identify useful marker genes with co-regulated expression. Sets of useful marker genes can be reduced to a smaller set, with each gene providing some non-redundant value in classification. This algorithm was used on the data set described in Alizadeh et al., supra, and the set of 380 informative gene markers was reduced to 234.

Supervised harvesting of expression trees (Hastie 2001) identifies genes or clusters that best distinguish one class from all the others on the data set. The method is used to identify the genes/clusters that can best separate one class versus all the others for datasets that include two or more classes or all classes from each other. This algorithm can be used for discovery or testing of a diagnostic gene set.

CART is a decision tree classification algorithm (Breiman 1984). From gene expression and or other data, CART can develop a decision tree for the classification of samples. Each node on the decision tree involves a query about the expression level of one or more genes or variables. Samples that are above the threshold go down one branch of the decision tree and samples that are not go down the other branch. See examples 10 and 16 for further description of its use in classification analysis and examples of its usefulness in discovering and implementing a diagnostic gene set. CART identifies surrogates for each splitter (genes that are the next best substitute for a useful gene in classification.

Multiple Additive Regression Trees (Friedman, JH 1999, MART) is similar to CART in that it is a classification algorithm that builds decision trees to distinguish groups. MART builds numerous trees for any classification problem and the resulting model involves a combination of the multiple trees. MART can select variables as it build models and thus can be used on large data sets, such as those derived from an 8000 gene microarray. Because MART uses a combination of many trees and does not take too much information from any one tree, it resists over training. MART identifies a set of genes and an algorithm for their use as a classifier.

A Nearest Shrunken Centroids Classifier can be applied to microarray or other data sets by the methods described by Tibshirani et al. 2002. This algorithms also identified gene sets for classification and determines their 10 fold cross validation error rates for each class of samples. The algorithm determines the error rates for models of any size, from one gene to all genes in the set. The error rates for either or both sample classes can are minimized when a particular number of genes are used. When this gene number is determined, the algorithm associated with the selected genes can be identified and employed as a classifier on prospective sample.

Once a set of genes and expression criteria for those genes have been established for classification, cross validation is done. There are many approaches, including a 10 fold cross validation analysis in which 10% of the training samples are left out of the analysis and the classification algorithm is built with the remaining 90%. The 10% are then used as a test set for the algorithm. The process is repeated 10 times with 10% of the samples being left out as a test set each time. Through this analysis, one can derive a cross validation error which helps estimate the robustness of the algorithm for use on prospective (test) samples.

Clinical data are gathered for every patient sample used for expression analysis. Clinical variables can be quantitative or non-quantitative. A clinical variable that is quantitiative can be used as a variable for significance or classification analysis. Non-quantitative clinical variables, such as the sex of the patient, can also be used in a significance analysis or classification analysis with some statistical tool. It is appreciated that the most useful diagnostic gene set for a condition may be optimal when considered along with one or more predictive clinical variables. Clinical data can also be used as supervising vectors for a correlation analysis. That is to say that the clinical data associated with each sample can be used to divide the samples into meaningful diagnostic categories for analysis. For example, samples can be divided into 2 or more groups based on the presence or absence of some diagnostic criterion (a). In addition, clinical data can be utilized to select patients for a correlation analysis or to exclude them based on some undesirable characteristic, such as an ongoing infection, a medicine or some other issue. Clincial data can also be used to assess the pre-test probability of an outcome. For example, patients who are female are much more likely to be diagnosed as having systemic lupus erythematosis than patients who are male.

Once a set of genes are identified that classify samples with acceptable accuracy. These genes are validated as a set using new samples that were not used to discover the gene set. These samples can be taken from frozen archieves from the discovery clinical study or can be taken from new patients prospectively. Validation using a "test set" of samples can be done using expression profiling of the gene set with microarrays or using real-time PCR for each gene on the test set samples. Alternatively, a different expression profiling technology can be used.

Validation and accuracy of diagnostic nucleotide sets

Prior to widespread application of the diagnostic probe sets of the invention the predictive value of the probe set is validated. When the diagnostic probe set is discovered by microarray based expression analysis, the differential expression of the member genes may be validated by a less variable and more quantitive and accurate technology such as real time PCR. In this type of experiment the amplification product is measured during the PCR reaction. This enables the researcher to observe the

amplification before any reagent becomes rate limiting for amplification. In kinetic PCR the measurement is of C_T (threshold cycle) or C_P (crossing point). This measurement (C_T=C_P) is the point at which an amplification curve crosses a threshold fluorescence value. The threshold is set to a point within the area where all of the reactions were in their linear phase of amplification. When measuring C_T, a lower C_T value is indicative of a higher amount of starting material since an earlier cycle number means the threshold was crossed more quickly.

Several fluorescence methodologies are available to measure amplification product in real-time PCR. Taqman (Applied BioSystems, Foster City, CA) uses fluorescence resonance energy transfer (FRET) to inhibit signal from a probe until the probe is degraded by the sequence specific binding and Taq 3′ exonuclease activity. Molecular Beacons (Stratagene, La Jolla, CA) also use FRET technology, whereby the fluorescence is measured when a hairpin structure is relaxed by the specific probe binding to the amplified DNA. The third commonly used chemistry is Sybr Green, a DNA-binding dye (Molecular Probes, Eugene, OR). The more amplified product that is produced, the higher the signal. The Sybr Green method is sensitive to non-specific amplification products, increasing the importance of primer design and selection. Other detection chemistries can also been used, such as ethedium bromide or other DNA-binding dyes and many modifications of the fluorescent dye/quencher dye Taqman chemistry, for example scorpions.

Real-time PCR validation can be done as described in Example 8.

Typically, the oligonucleotide sequence of each probe is confirmed, e.g. by DNA sequencing using an oligonucleotide-specific primer. Partial sequence obtained is generally sufficient to confirm the identity of the oligonucleotide probe. Alternatively, a complementary polynucleotide is fluorescently labeled and hybridized to the array, or to a different array containing a resynthesized version of the oligo nucleotide probe, and detection of the correct probe is confirmed.

Typically, validation is performed by statistically evaluating the accuracy of the correspondence between the molecular signature for a diagnostic probe set and a selected indicator. For example, the expression differential for a nucleotide sequence between two subject classes can be expressed as a simple ratio of relative expression. The expression of the nucleotide sequence in subjects with selected indicator can be compared to the expression of that nucleotide sequence in subjects without the indicator, as described in the following equations.

$\sum E_x ai/N = E_x A$ the average expression of nucleotide sequence x in the members of group A;

$\sum E_x bi/M = E_x B$ the average expression of nucleotide sequence x in the members of group B;

$E_x A/ ExB = \Delta E_x AB$ the average differential expression of nucleotide sequence x between groups A and B:

where $\sum$ indicates a sum; Ex is the expression of nucleotide sequence x relative to a standard; ai are the individual members of group A, group A has N members; bi are the individual members of group B, group B has M members.

Individual components of a diagnostic probe set each have a defined sensitivity and specificity for distinguishing between subject groups. Such individual nucleotide sequences can be employed in concert as a diagnostic probe set to increase the sensitivity and specificity of the evaluation. The database of molecular signatures is queried by algorithms to identify the set of nucleotide sequences (i.e., corresponding to members of the probe set) with the highest average differential expression between subject groups. Typically, as the number of nucleotide sequences in the diagnostic probe set increases, so does the predictive value, that is, the sensitivity and specificity of the probe set. When the probe sets are defined they may be used for diagnosis and patient monitoring as discussed below. The diagnostic sensitivity and specificity of the probe sets for the defined use can be determined for a given probe set with specified expression levels as demonstrated above. By altering the expression threshold required for the use of each nucleotide sequence as a diagnostic, the sensitivity and specificity of the probe set can be altered by the practitioner. For example, by lowering the magnitude of the expression differential threshold for each nucleotide sequence in the set, the sensitivity of the test will increase, but the specificity will decrease. As is apparent from the foregoing discussion, sensitivity and specificity are inversely related and the predictive accuracy of the probe set is continuous and dependent on the expression threshold set for each nucleotide sequence. Although sensitivity and specificity tend to have an inverse relationship when expression thresholds are altered, both parameters can be increased as nucleotide sequences with predictive value are added to the diagnostic nucleotide set. In addition a single or a few markers may not be reliable expression markers across a population of patients. This is because of the variability in expression and measurement of expression that exists between measurements, individuals and individuals over time. Inclusion of a large number of candidate nucleotide sequences or large numbers of nucleotide sequences in a diagnostic nucleotide set allows for this variability as not all nucleotide sequences need to meet a threshold for diagnosis. Generally, more markers are better than a single marker. If many markers are used to make a diagnosis, the likelihood that all expression markers will not meet some thresholds based upon random variability is low and thus the test will give fewer false negatives. Surrogate markers are useful for these purposes. These are markers or genes that are coordinately expressed. Surrogate markers essential provide redundant infomation, but this redundancy can improve accuracy by decreasing errors due to assay variability.

It is appreciated that the desired diagnostic sensitivity and specificity of the diagnostic nucleotide set may vary depending on the intended use of the set. For example, in certain uses, high specificity and high sensitivity are desired. For example, a diagnostic nucleotide set for predicting which patient population may experience side effects may require high sensitivity so as to avoid treating such patients. In other settings, high sensitivity is desired, while reduced specificity may be tolerated. For example, in the case of a beneficial treatment with few side effects, it may be important to identify as many patients as possible (high sensitivity) who will respond to the drug, and treatment of some patients who will not respond is tolerated. In other settings, high specificity is desired and reduced sensitivity may be tolerated. For example, when identifying patients for an early-phase clinical trial, it is important to identify patients who may respond to the particular treatment. Lower sensitivity is tolerated in this setting as it merely results in reduced patients who enroll in the study or requires that more patients are screened for enrollment.

To discover and validate a gene set that can be applied to accurately diagnose or classify patients across the country or around the world, it is necessary to ensure that the gene set was developed and validated using samples that represent the types of patients that will be encountered in the clinical setting. For example, diverse ethnicity, drug usage and clinical practice patterns must all be represented in the discovery and validation to ensure that the test works on this variety of patients.

Immune Monitoring

Leukocyte gene expression can be used to monitor the immune system. Immune monitoring examines both the level of gene expression for a set of genes in a given cell type and for genes which are expressed in a cell type selective manner gene expression monitoring will also detect the presence or absence of new cell types, progenitor cells, differentiation of cells and the like. Gene expression patterns may be associated with activation or the resting state of cells of the immune system that are responsible for or responsive to a disease state. For example, in the process of lupus and other autoimmune diseases, cells of the immune system are activated by self-antigens. Genes and gene sets that monitor and diagnose this process are providing a measure of the level and type of activation of the immune system. Genes and gene sets that are useful in monitoring the immune system may be useful for diagnosis and monitoring of all diseases that involve the immune system. Some examples are rheumatoid arthritis, lupus, inflammatory bowel diseases, multiple sclerosis, HIV/AIDS, and viral, bacterial and fungal infection. All disorders and diseases disclosed herein are contemplated. Genes and gene sets that monitor immune activation are useful for monitoring response to immunosuppressive drug therapy, which is used to decrease immune activation. Genes are found to correlate with immune activation by correlation of expression patterns to the known presence of immune activation or quiescence in a sample as determined by some other test.

Selected Diseases

In principle, individual oligonucleotides and diagnostic oligonucleotide sets of the invention may be developed and applied to essentially any disease, or disease criterion, as long as at least one subset of oligonucleotide sequences is differentially expressed in samples derived from one or more individuals with a disease criteria or disease and one or more individuals without the disease criteria or disease, wherein the individual may be the same individual sampled at different points in time, or the individuals may be different individuals (or populations of individuals). For example, the subset of oligonucleotide sequences may be differentially expressed in the sampled tissues of subjects with the disease or disease criterion (e.g., a patient with a disease or disease criteria) as compared to subjects without the disease or disease criterion (e.g., patients without a disease (control patients)). Alternatively, or in addition, the subset of oligonucleotide sequence(s) may be differentially expressed in different samples taken from the same patient, e.g at different points in time, at different disease stages, before and after a treatment, in the presence or absence of a risk factor, etc.

Expression profiles corresponding to oligonucleotides and sets of oligonucleotide sequences that correlate not with a diagnosis, but rather with a particular aspect of a disease can also be used to identify the diagnostic oligonucleotide sets and disease specific target oligonucleotide sequences of the invention. For example, such an aspect, or disease criterion, can relate to a subject's medical or family

history, e.g., occurance of an autoimmune disease, childhood illness, cause of death of a parent or other relative, prior surgery or other intervention, medications, laboratory values and results of diagnostic testing (radiology, pathology, etc.), symptoms (including onset and/or duration of symptoms), etc. Alternatively, the disease criterion can relate to a diagnosis, e.g., chronic inflammatory disease such as lupus, rheumatoid arthritis, osteoarthritis, or prognosis (e.g., prediction of future diagnoses, events or complications), e.g., renal failure from lupus, joint replacement surgery for rheumatoid arthritis, rheumatoid arthritis or systemic lupus erythematosis disease activity or the like. In other cases, the disease criterion corresponds to a therapeutic outcome, e.g., response to a medication, response to a surgery or physical therapy for a joint. Alternatively, the disease criteria correspond with previously identified or classic risk factors and may correspond to prognosis or future disease diagnosis. As indicated above, a disease criterion can also correspond to genotype for one or more loci. Disease criteria (including patient data) may be collected (and compared) from the same patient at different points in time, from different patients, between patients with a disease (criterion) and patients respresenting a control population, etc. Longitudinal data, i.e., data collected at different time points from an individual (or group of individuals) may be used for comparisons of samples obtained from an individual (group of individuals) at different points in time, to permit identification of differences specifically related to the disease state, and to obtain information relating to the change in expression over time, including a rate of change or trajectory of expression over time. The usefulness of longitudinal data is further discussed in the section titled "Identification of diagnostic nucleotide sets of the invention".

It is further understood that diagnostic oligonucleotides and oligonucleotide sets may be developed for use in diagnosing conditions for which there is no present means of diagnosis. For example, in rheumatoid arthritis, joint destruction is often well under way before a patient experience symptoms of the condition. A diagnostic nucleotide or nucleotide set may be developed that diagnoses rheumatic joint destruction at an earlier stage than would be possible using present means of diagnosis, which rely in part on the presentation of symptoms by a patient. Diagnostic nucleotide sets may also be developed to replace or augment current diagnostic procedures. For example, the use of a diagnostic nucleotide or nucleotide set to diagnose lupus may replace or supplement the current diagnostic tests and strategies.

It is understood that the following discussion of diseases is exemplary and non-limiting, and further that the general criteria discussed above, e.g. use of family medical history, are generally applicable to the specific diseases discussed below.

In addition to leukocytes, as described throughout, the general method is applicable to oligonucleotide sequences that are differentially expressed in any subject tissue or cell type, by the collection and assessment of samples of that tissue or cell type. However, in many cases, collection of such samples presents significant technical or medical problems given the current state of the art.

Systemic Lupus Erythematosis (SLE)

SLE is a chronic, systemic inflammatory disease characterized by dysregulation of the immune system, which effects up to 2 million patients in the US. Symptoms of SLE include rashes, joint pain, abnormal blood counts, renal dysfunction and damage, infections, CNS disorders, arthralgias

and autoimmunity. Patients may also have early onset atherosclerosis. The diagnosis of SLE is difficult to make with certainty using current diagnostic tests and algorithms. Antibody tests can be specific for the disease, but often lack sensitivity. Clinical diagnosis may lack both high sensisivity and specificity. SLE is a disease that clearly involves differential gene expression in leukocytes compared to patients without the disease.

Diagnostic oligonucleotides and oligonucleotide sets are identified and validated for use in diagnosis and monitoring of SLE activity and progression. Disease criteria correspond to clinical data, e.g. symptom rash, joint pain, malaise, rashes, blood counts (white and red), tests of renal function e.g. creatinine, blood urea nitrogen (hereinafter, "bun") creative clearance, data obtained from laboratory tests, including complete blood counts with differentials, CRP, ESR, ANA, Serum IL6, Soluble CD40 ligand, LDL, HDL, Anti-DNA antibodies, rheumatoid factor, C3, C4, serum creatinine and any medication levels, the need for pain medications, cumulative doses or immunosuppressive therapy, symptoms or any manifestation of carotid atherosclerosis (e.g. ultrasound diagnosis or any other manifestations of the disease), data from surgical procedures such as gross operative findings and pathological evaluation of resected tissues and biopsies (e.g., renal, CNS), information on pharmacological therapy and treatment changes, clinical diagnoses of disease "flare", hospitalizations, death, response to medications, quantitative joint exams, results from health assessment questionnaires (HAQs), and other clinical measures of patient symptoms and disability. In addition, disease criteria correspond to the clinical score known as SLEDAI (Bombadier C, Gladman DD, Urowitz MB, Caron D, Chang CH and the Committee on Prognosis Studies in SLE: Derivation of the SLEDAI for Lupus Patients. Arthritis Rheum 35:630-640, 1992.). Diagnostic nucleotide sets may be useful for diagnosis of SLE, monitoring disease progression including progressive renal dysfunction, carotid atherosclerosis and CNS dysfunction, and predicting occurrence of side-effects, for example.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) effects about two million patients in the US and is a chronic and debilitating inflammatory arthritis, particularly involving pain and destruction of the joints. RA often goes undiagnosed because patients may have no pain, but the disease is actively destroying the joint. Other patients are known to have RA, and are treated to alleviate symptoms, but the rate of progression of joint destruction can't easily be monitored. Drug therapy is available, but the most effective medicines are toxic (e.g., steroids, methotrexate) and thus need to be used with caution. A new class of medications (TNF blockers) is very effective, but the drugs are expensive, have side effects, and not all patients respond. Side-effects are common and include immune suppression, toxicity to organ systems, allergy and metabolic disturbances.

Diagnostic oligonucleotides and oligonucleotide sets of the invention are developed and validated for use in diagnosis and treatment of RA. Disease criteria correspond to disease symptoms (e.g., joint pain, joint swelling and joint stiffness and any of the American College for Rheumatology criteria for the diagnosis of RA, see Arnett et al (1988) Arthr. Rheum. 31:315-24), progression of joint destruction (e.g. as measured by serial hand radiographs, assessment of joint function and mobility), surgery, need for medication, additional diagnoses of inflammatory and non-inflammatory conditions, and clinical laboratory measurements including complete blood counts with differentials, CRP, ESR,

ANA, Serum IL6, Soluble CD40 ligand, LDL, HDL, Anti-DNA antibodies, rheumatoid factor, C3, C4, serum creatinine, death, hospitalization and disability due to joint destruction. In addition, or alternatively, disease criteria correspond to response to drug therapy and presence or absence of side-effects or measures of improvement exemplified by the American College of Rheumatology "20%" and "50%" response/improvement rates. See Felson et al (1995) Arthr Rheum 38:531-37. Diagnostic nucleotide sets are identified that monitor and predict disease progression including flaring (acute worsening of disease accompanied by joint pain or other symptoms), response to drug treatment and likelihood of side-effects.

In addition to peripheral leukocytes, surgical specimens of rheumatoid joints can be used for leukocyte expression profiling experiments. Members of diagnostic nucleotide sets are candidates for leukocyte target nucleotide sequences, e.g. as a candidate drug target for rheumatoid arthritis. Synovial specimens can be used for expression profiling or cells derived and sorted from that tissue (such as subsets of leukocytes) can be used. Cells can be separated by fluorescence activated cell sorting or magnetic affinity reagent techniques or some other technique. Synovial specimens and blood can be obtained from the same patient and gene expression can be compared between these 2 sample types.

Osteoarthritis

20-40 million patients in the US have osteoarthritis. Patient groups are heterogeneous, with a subset of patients having earlier onset, more aggressive joint damage, involving more inflammation (leukocyte infiltration). Leukocyte diagnostics can be used to distinguish osteoarthritis from rheumatoid arthritis and other differntial diagnoses, define likelihood and degree of response to NSAID therapy (non-steroidal anti-inflammatory drugs) or other anti-inflammatory therapies. Rate of progression of joint damage can also be assessed. Diagnostic nucleotide sets may be developed for use in selection and titration of treatment therapies. Disease criteria correspond to response to therapy, and disease progression using certain therapies, response to medications, need for joint surgery, joint pain and disability.

In addition to peripheral leukocytes, surgical specimens of osteoarthritic joints can be used for leukocyte expression profiling experiments. Diagnostic oligonucleotides and diagnostic oligonucleotide sets are candidates for leukocyte target nucleotide sequences, e.g. as a candidate drug target for osteoarthritis. Synovial specimens can be used for expression profiling or cells derived and sorted from that tissue (such as subsets of leukocytes) can be used. Cells can be separated by fluorescence activated cell sorting or magnetic affinity reagent techniques or some other technique. Synovial specimens and blood can be obtained from the same patient and gene expression can be compared between these 2 sample types.

In another example, diagnostic nucleotide sets are developed and validated for use in diagnosis and therapy of peri-prosthetic osteolysis. In this disease, a prosthetic joint such as a knee or hip is found to loosen over time and requires repeat surgery. Loosening may occur in some patients due to an inflammatory response incited by the foreign material of the prosthesis. Disease criteria include joint loosening, radiographic evidence of peri-prosthetic osteolysis, need for repeat surgery, response to pharmacological therapy, and/or histological (from biopsy or surgery) or biochemical (markers of bone metabolism such as alkaline phosphatase) evidence of osteolysis. Tissues used for

expression profiling can include peripheral leukocytes or leukocyte subsets, periprosthetic tissue, or synovial fluid. In addition, gene sets can be discovered using an *in vitro*model of the disease in which immune cells are exposed to prosthesis materials such as cement or titanium.

Pharmacogenomics

Pharmocogenomics is the study of the individual propensity to respond to a particular drug therapy (combination of therapies). In this context, response can mean whether a particular drug will work on a particular patient, e.g. some patients respond to one drug but not to another drug. One example of this would be prediction of a patient's response to drugs that target IFNs. Response can also refer to the likelihood of successful treatment or the assessment of progress in treatment. Titration of drug therapy to a particular patient is also included in this description, e.g. different patients can respond to different doses of a given medication. This aspect may be important when drugs with side-effects or interactions with other drug therapies are contemplated.

Diagnostic oligonucleotides and oligonucleotide sets are developed and validated for use in assessing whether a patient will respond to a particular therapy and/or monitoring response of a patient to drug therapy (therapies). Disease criteria correspond to presence or absence of clinical symptoms or clinical endpoints, presence of side-effects or interaction with other drug(s). The diagnostic nucleotide set may further comprise nucleotide sequences that are targets of drug treatment or markers of active disease.

Diagnostic oligonucleotides and oligonucleotide sets are developed and validated for use in assessing whether a patient has a particular drug toxicity or toxicity due to an environmental, work-related or other agent. Such exposures of the patient may also be related to biological or biochemical agents used in warfare. Diagnostic oligonucleotides and oligonucleotide sets may allow early diagnosis of a toxicity or exposure or may monitor the severity and course of toxic responses.

Methods of using diagnostic oligonucleotides and oligonucleotide sets.

The invention also provide methods of using the diagnostic oligonucleotides and oligonucleotide sets to: diagnose or monitor disease; assess severity of disease; predict future occurrence of disease; predict future complications of disease; determine disease prognosis; evaluate the patient's risk, or "stratify" a group of patients; assess response to current drug therapy; assess response to current non-pharmacological therapy; determine the most appropriate medication or treatment for the patient; predict whether a patient is likely to respond to a particular drug; and determine most appropriate additional diagnostic testing for the patient, among other clinically and epidemiologically relevant applications.

The oligonucleotides and oligonucleotide sets of the invention can be utilized for a variety of purposes by physicians, healthcare workers, hospitals, laboratories, patients, companies and other institutions. As indicated previously, essentially any disease, condition, or status for which at least one nucleotide sequence is differentially expressed in leukocyte populations (or sub-populations) can be evaluated, e.g., diagnosed, monitored, etc. using the diagnostic nucleotide sets and methods of the invention. In addition to assessing health status at an individual level, the diagnostic nucleotide sets of

the present invention are suitable for evaluating subjects at a "population level," e.g., for epidemiological studies, or for population screening for a condition or disease.

Collection and preparation of sample

RNA, protein and/or DNA are prepared using methods well-known in the art, as further described herein. It is appreciated that subject samples collected for use in the methods of the invention are generally collected in a clinical setting, where delays may be introduced before RNA samples are prepared from the subject samples of whole blood, e.g. the blood sample may not be promptly delivered to the clinical lab for further processing. Further delay may be introduced in the clinical lab setting where multiple samples are generally being processed at any given time. For this reason, methods that feature lengthy incubations of intact leukocytes at room temperature are not preferred, because the expression profile of the leukocytes may change during this extended time period. For example, RNA can be isolated from whole blood using a phenol/guanidine isothiocyanate reagent or another direct whole-blood lysis method, as described in, e.g., U.S. Patent Nos. 5,346,994 and 4,843,155. This method may be less preferred under certain circumstances because the large majority of the RNA recovered from whole blood RNA extraction comes from erythrocytes since these cells outnumber leukocytes 1000:1. Care must be taken to ensure that the presence of erythrocyte RNA and protein does not introduce bias in the RNA expression profile data or lead to inadequate sensitivity or specificity of probes.

Alternatively, intact leukocytes may be collected from whole blood using a lysis buffer that selectively lyses erythrocytes, but not leukocytes, as described, e.g., in (U.S. Patent Nos. 5,973,137, and 6,020,186). Intact leukocytes are then collected by centrifugation, and leukocyte RNA is isolated using standard protocols, as described herein. However, this method does not allow isolation of sub-populations of leukocytes, e.g. mononuclear cells, which may be desired. In addition, the expression profile may change during the lengthy incubation in lysis buffer, especially in a busy clinical lab where large numbers of samples are being prepared at any given time.

Alternatively, specific leukocyte cell types can be separated using density gradient reagents (Boyum, A, 1968.). For example, mononuclear cells may be separated from whole blood using density gradient centrifugation, as described, e.g., in U.S. Patents Nos. 4190535, 4350593, 4751001, 4818418, and 5053134. Blood is drawn directly into a tube containing an anticoagulant and a density reagent (such as Ficoll or Percoll). Centrifugation of this tube results in separation of blood into an erythrocyte and granulocyte layer, a mononuclear cell suspension, and a plasma layer. The mononuclear cell layer is easily removed and the cells can be collected by centrifugation, lysed, and frozen. Frozen samples are stable until RNA can be isolated. Density centrifugation, however, must be conducted at room temperature, and if processing is unduly lengthy, such as in a busy clinical lab, the expression profile may change.

Alternatively, cells can be separated using fluorescence activated cell sorting (FACS) or some other technique, which divides cells into subsets based on gene or protein expression. This may be desirable to enrich the sample for cells of interest, but it may also introduce cell manipulations and time delays, which result in alteration of gene expression profiles (Cantor et al. 1975; Galbraith et al. 1999).

The quality and quantity of each clinical RNA sample is desirably checked before amplification and labeling for array hybridization, using methods known in the art. For example, one microliter of each sample may be analyzed on a Bioanalyzer (Agilent 2100 Palo Alto, CA. USA) using an RNA 6000 nano LabChip (Caliper, Mountain View, CA. USA). Degraded RNA is identified by the reduction of the 28S to 18S ribosomal RNA ratio and/or the presence of large quantities of RNA in the 25-100 nucleotide range.

It is appreciated that the RNA sample for use with a diagnostic oligonucleotide or oligonucleotide set may be produced from the same or a different cell population, sub-population and/or cell type as used to identify the diagnostic nucleotide set. For example, a diagnostic oligonucleotide or oligonucleotide set identified using RNA extracted from mononuclear cells may be suitable for analysis of RNA extracted from whole blood or mononuclear cells, depending on the particular characteristics of the members of the diagnostic nucleotide set. Generally, diagnostic oligonucleotides or oligonucleotide sets must be tested and validated when used with RNA derived from a different cell population, sub-population or cell type than that used when obtaining the diagnostic gene set. Factors such as the cell-specific gene expression of diagnostic nucleotide set members, redundancy of the information provided by members of the diagnostic nucleotide set, expression level of the member of the diagnostic nucleotide set, and cell-specific alteration of expression of a member of the diagnostic nucleotide set will contribute to the usefullness of a different RNA source than that used when identifying the members of the diagnostic nucleotide set. It is appreciated that it may be desirable to assay RNA derived from whole blood, obviating the need to isolate particular cell types from the blood.

Rapid method of RNA extraction suitable for production in a clinical setting of high quality RNA for expression profiling

In a clinical setting, obtaining high quality RNA preparations suitable for expression profiling, from a desired population of leukocytes poses certain technical challenges, including: the lack of capacity for rapid, high-throughput sample processing in the clinical setting, and the possibility that delay in processing (in a busy lab or in the clinical setting) may adversely affect RNA quality, e.g. by a permitting the expression profile of certain nucleotide sequences to shift. Also, use of toxic and expensive reagents, such as phenol, may be disfavored in the clinical setting due to the added expense associated with shipping and handling such reagents.

A useful method for RNA isolation for leukocyte expression profiling would allow the isolation of monocyte and lymphocyte RNA in a timely manner, while preserving the expression profiles of the cells, and allowing inexpensive production of reproducible high-quality RNA samples. Accordingly, the invention provides a method of adding inhibitor(s) of RNA transcription and/or inhibitor(s) of protein synthesis, such that the expression profile is "frozen" and RNA degradation is reduced. A desired leukocyte population or sub-population is then isolated, and the sample may be frozen or lysed before further processing to extract the RNA. Blood is drawn from subject population and exposed to ActinomycinD (to a final concentration of 10 ug/ml) to inhibit transcription, and cycloheximide (to a final concentration of 10 ug/ml) to inhibit protein synthesis. The inhibitor(s) can be injected into the blood collection tube in liquid form as soon as the blood is drawn, or the tube can

be manufactured to contain either lyophilized inhibitors or inhibitors that are in solution with the anticoagulant. At this point, the blood sample can be stored at room temperature until the desired leukocyte population or sub-population is isolated, as described elsewhere. RNA is isolated using standard methods, e.g., as described above, or a cell pellet or extract can be frozen until further processing of RNA is convenient.

The invention also provides a method of using a low-temperature density gradient for separation of a desired leukocyte sample. In another embodiment, the invention provides the combination of use of a low-temperature density gradient and the use of transcriptional and/or protein synthesis inhibitor(s). A desired leukocyte population is separated using a density gradient solution for cell separation that maintains the required density and viscosity for cell separation at 0-4□C. Blood is drawn into a tube containing this solution and may be refrigerated before and during processing as the low temperatures slow cellular processes and minimize expression profile changes. Leukocytes are separated, and RNA is isolated using standard methods. Alternately, a cell pellet or extract is frozen until further processing of RNA is convenient. Care must be taken to avoid rewarming the sample during further processing steps.

Alternatively, the invention provides a method of using low-temperature density gradient separation, combined with the use of actinomycin A and cyclohexamide, as described above.

Assessing expression for diagnostics

Expression profiles for the oligonucleotides or the set of diagnostic oligonucleotide sequences in a subject sample can be evaluated by any technique that determines the expression of each component oligonucleotide sequence. Methods suitable for expression analysis are known in the art, and numerous examples are discussed in the Sections titled "Methods of obtaining expression data" and "high throughput expression Assays", above.

In many cases, evaluation of expression profiles is most efficiently, and cost effectively, performed by analyzing RNA expression. Alternatively, the proteins encoded by each component of the diagnostic nucleotide set are detected for diagnostic purposes by any technique capable of determining protein expression, e.g., as described above. Expression profiles can be assessed in subject leukocyte sample using the same or different techniques as those used to identify and validate the diagnostic oligonucleotide or oligonucleotide set. For example, a diagnostic nucleotide set identified as a subset of sequences on a cDNA microarray can be utilized for diagnostic (or prognostic, or monitoring, etc.) purposes on the same array from which they were identified. Alternatively, the diagnostic nucleotide sets for a given disease or condition can be organized onto a dedicated sub-array for the indicated purpose. It is important to note that if diagnostic nucleotide sets are discovered using one technology, e.g. RNA expression profiling, but applied as a diagnostic using another technology, e.g. protein expression profiling, the nucleotide (or gene, or protein) sets must generally be validated for diagnostic purposes with the new technology. In addition, it is appreciated that diagnostic nucleotide sets that are developed for one use, e.g. to diagnose a particular disease, may later be found to be useful for a different application, e.g. to predict the likelihood that the particular disease will occur. Generally, the diagnostic nucleotide set will need to be validated for use in the second circumstance. As discussed herein, the sequence of diagnostic nucleotide set members may be

amplified from RNA or cDNA using methods known in the art providing specific amplification of the nucleotide sequences.

General Protein Methods

Protein products of the nucleotide sequences of the invention may include proteins that represent functionally equivalent gene products. Such an equivalent gene product may contain deletions, additions or substitutions of amino acid residues within the amino acid sequence encoded by the nucleotide sequences described, above, but which result in a silent change, thus producing a functionally equivalent nucleotide sequence product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Functionally equivalent", as utilized herein, refers to a protein capable of exhibiting a substantially similar in vivo activity as the endogenous gene products encoded by the nucleotides described herein.

The gene products (protein products of the nucleotide sequences) may be produced by recombinant DNA technology using techniques well known in the art. Methods which are well known to those skilled in the art can be used to construct expression vectors containing novel nucleotide sequence protein coding sequences and appropriate transcriptional/translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Sambrook et al., 1989, supra, and Ausubel et al., 1989, supra. Alternatively, RNA capable of encoding novel nucleotide sequence protein sequences may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, M. J. ed., IRL Press, Oxford, which is incorporated by reference herein in its entirety.

A variety of host-expression vector systems may be utilized to express the nucleotide sequence coding sequences of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, exhibit the protein encoded by the nucleotide sequence of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing nucleotide sequence protein coding sequences; yeast (e.g. Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing the nucleotide sequence protein coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the nucleotide sequence protein coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing nucleotide sequence protein

coding sequences; or mammalian cell systems (e.g. COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5 K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the nucleotide sequence protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of antibodies or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791;), in which the nucleotide sequence protein coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503;-5509); and the likes of pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target nucleotide sequence protein can be released from the GST moiety. Other systems useful in the invention include use of the FLAG epitope or the 6-HIS systems.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign nucleotide sequences. The virus grows in Spodoptera frugiperda cells. The nucleotide sequence coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of nucleotide sequence coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted nucleotide sequence is expressed. (E.g., see Smith et al., 1983, J. Virol. 46: 584; Smith, U.S. Pat. No. 4,215,051;).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the nucleotide sequence coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric nucleotide sequence may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing nucleotide sequence encoded protein in infected hosts. (E.g., See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:3655-3659;). Specific initiation signals may also be required for efficient translation of inserted nucleotide sequence coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire nucleotide sequence, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the

nucleotide sequence coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:516-544;).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the product of the nucleotide sequence in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, etc.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the nucleotide sequence encoded protein may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express nucleotide sequence encoded protein. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the nucleotide sequence encoded protein.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223;), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026;), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817;) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527;); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072;); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1;); and hygro, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30: 147 ; 147) genes.

An alternative fusion protein system allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, et al., 1991, Proc. Natl. Acad. Sci. USA 88: 8972-8976). In this system, the nucleotide sequence of interest is subcloned into a vaccinia recombination plasmid such that the nucleotide sequence's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni.sup.2 +-nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

Where recombinant DNA technology is used to produce the protein encoded by the nucleotide sequence for such assay systems, it may be advantageous to engineer fusion proteins that can facilitate labeling, immobilization and/or detection.

Antibodies

Indirect labeling involves the use of a protein, such as a labeled antibody, which specifically binds to the protein encoded by the nucleotide sequence. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression library.

The invention also provides for antibodies to the protein encoded by the nucleotide sequences. Described herein are methods for the production of antibodies capable of specifically recognizing one or more nucleotide sequence epitopes. Such antibodies may include, but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')2 fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Such antibodies may be used, for example, in the detection of a nucleotide sequence in a biological sample, or, alternatively, as a method for the inhibition of abnormal gene activity, for example, the inhibition of a disease target nucleotide sequence, as further described below. Thus, such antibodies may be utilized as part of cardiovascular or other disease treatment method, and/or may be used as part of diagnostic techniques whereby patients may be tested for abnormal levels of nucleotide sequence encoded proteins, or for the presence of abnormal forms of the such proteins.

For the production of antibodies to a nucleotide sequence, various host animals may be immunized by injection with a protein encoded by the nucleotide sequence, or a portion thereof. Such host animals may include but are not limited to rabbits, mice, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals such as those described above, may be immunized by injection with gene product supplemented with adjuvants as also described above.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and Milstein, (1975, Nature 256:495-497; and U.S. Pat. No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) can be adapted to produce nucleotide sequence-single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments which recognize specific epitopes may be generated by known techniques For example, such fragments include but are not limited to: the F(ab')2 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Disease specific target oligonucleotide sequences

The invention also provides disease specific target oligonucleotide sequences, and sets of disease specific target oligonucleotide sequences. The diagnostic oligonucleotide sets, subsets thereof, novel oligonucleotide sequences, and individual members of the diagnostic oligonucleotide sets identified as described above are also disease specific target oligonucleotide sequences. In particular, individual oligonucleotide sequences that are differentially regulated or have predictive value that is strongly correlated with a disease or disease criterion are especially favorable as disease specific target oligonucleotide sequences. Sets of genes that are co-regulated may also be identified as disease specific target oligonucleotide sets. Such oligonucleotide sequences and/or oligonucleotide sequence products are targets for modulation by a variety of agents and techniques. For example, disease specific target oligonucleotide sequences (or the products of such oligonucleotide sequences, or sets of disease specific target oligonucleotide sequences) can be inhibited or activated by, e.g., target specific monoclonal antibodies or small molecule inhibitors, or delivery of the oligonucleotide sequence or

gene product of the oligonucleotide sequence to patients. Also, sets of genes can be inhibited or activated by a variety of agents and techniques. The specific usefulness of the target oligonucleotide sequence(s) depends on the subject groups from which they were discovered, and the disease or disease criterion with which they correlate.

Identification of nucleotide sequence involved in leukocyte adhesion

The invention also encompasses a method of identifying nucleotide sequences involved in leukocyte adhesion. The interaction between the endothelial cell and leukocyte is a fundamental mechanism of all inflammatory disorders, including the diseases listed in Table 3. For example, the first visible abnormality in atherosclerosis is the adhesion to the endothelium and diapedesis of mononuclear cells (e.g., T-cell and monocyte). Insults to the endothelium (for example, cytokines, tobacco, diabetes, hypertension and many more) lead to endothelial cell activation. The endothelium then expresses adhesion molecules, which have counter receptors on mononuclear cells. Once the leukocyte receptors have bound the endothelial adhesion molecules, they stick to the endothelium, roll a short distance, stop and transmigrate across the endothelium. A similar set of events occurs in both acute and chronic inflammation. When the leukocyte binds the endothelial adhesion molecule, or to soluble cytokines secreted by endothelial or other cells, a program of gene expression is activated in the leukocyte. This program of expression leads to leukocyte rolling, firm adhesion and transmigration into the vessel wall or tissue parenchyma. Inhibition of this process is highly desirable goal in anti-inflammatory drug development. In addition, leukocyte nucleotide sequences and epithelial cell nucleotide sequences, that are differentially expressed during this process may be disease-specific target nucleotide sequences.

Human endothelial cells, e.g. derived from human coronary arteries, human aorta, human pulmonary artery, human umbilical vein or microvascular endothelial cells, are cultured as a confluent monolayer, using standard methods. Some of the endothelial cells are then exposed to cytokines or another activating stimuli such as oxidized LDL, hyperglycemia, shear stress, or hypoxia (Moser et al. 1992). Some endothelial cells are not exposed to such stimuli and serve as controls. For example, the endothelial cell monolayer is incubated with culture medium containing 5 U/ml of human recombinant IL-1alpha or 10 ng/ml TNF (tumor necrosis factor), for a period of minutes to overnight. The culture medium composition is changed or the flask is sealed to induce hypoxia. In addition, tissue culture plate is rotated to induce sheer stress.

Human T-cells and/or monocytes are cultured in tissue culture flasks or plates, with LGM-3 media from Clonetics. Cells are incubated at 37 degree C, 5% CO_2 and 95% humidity. These leukocytes are exposed to the activated or control endothelial layer by adding a suspension of leukocytes on to the endothelial cell monolayer. The endothelial cell monolayer is cultured on a tissue culture treated plate/ flask or on a microporous membrane. After a variable duration of exposures, the endothelial cells and leukocytes are harvested separately by treating all cells with trypsin and then sorting the endothelial cells from the leukocytes by magnetic affinity reagents to an endothelial cell specific marker such as PECAM-1 (Stem Cell Technologies). RNA is extracted from the isolated cells by standard techniques. Leukocyte RNA is labeled as described above, and hybridized to leukocyte candidate nucleotide library. Epithelial cell RNA is also labeled and hybridized to the leukocyte

candidate nucleotide library. Alternatively, the epithelial cell RNA is hybridized to a epithelial cell candidate nucleotide library, prepared according to the methods described for leukocyte candidate libraries, above.

Hybridization to candidate nucleotide libraries will reveal nucleotide sequences that are up-regulated or down-regulated in leukocyte and/or epithelial cells undergoing adhesion. The differentially regulated nucleotide sequences are further characterized, e.g. by isolating and sequencing the full-length sequence, analysis of the DNA and predicted protein sequence, and functional characterization of the protein product of the nucleotide sequence, as described above. Further characterization may result in the identification of leukocyte adhesion specific target nucleotide sequences, which may be candidate targets for regulation of the inflammatory process. Small molecule or antibody inhibitors can be developed to inhibit the target nucleotide sequence function. Such inhibitors are tested for their ability to inhibit leukocyte adhesion in the in vitro test described above.

Integrated systems

Integrated systems for the collection and analysis of expression profiles, and molecular signatures, as well as for the compilation, storage and access of the databases of the invention, typically include a digital computer with software including an instruction set for sequence searching and analysis, and, optionally, high-throughput liquid control software, image analysis software, data interpretation software, a robotic control armature for transferring solutions from a source to a destination (such as a detection device) operably linked to the digital computer, an input device (e.g., a computer keyboard) for entering subject data to the digital computer, or to control analysis operations or high throughput sample transfer by the robotic control armature. Optionally, the integrated system further comprises an image scanner for digitizing label signals from labeled assay components, e.g., labeled nucleic acid hybridized to a candidate library microarray. The image scanner can interface with image analysis software to provide a measurement of the presence or intensity of the hybridized label, i.e., indicative of an on/off expression pattern or an increase or decrease in expression.

Readily available computational hardware resources using standard operating systems are fully adequate, e.g., a PC (Intel x86 or Pentium chip- compatible DOS,™ OS2,™ WINDOWS,™ WINDOWS NT,™ WINDOWS95,™ WINDOWS98,™ LINUX, or even Macintosh, Sun or PCs will suffice) for use in the integrated systems of the invention. Current art in software technology is similarly adequate (i.e., there are a multitude of mature programming languages and source code suppliers) for design, e.g., of an upgradeable open-architecture object-oriented heuristic algorithm, or instruction set for expression analysis, as described herein. For example, software for aligning or otherwise manipulating molecular signatures can be constructed by one of skill using a standard programming language such as Visual basic, Fortran, Basic, Java, or the like, according to the methods herein.

Various methods and algorithms, including genetic algorithms and neural networks, can be used to perform the data collection, correlation, and storage functions, as well as other desirable functions, as described herein. In addition, digital or analog systems such as digital or analog computer systems can control a variety of other functions such as the display and/or control of input and output files.

For example, standard desktop applications such as word processing software (e.g., Corel WordPerfect™ or Microsoft Word™) and database software (e.g., spreadsheet software such as Corel Quattro Pro™, Microsoft Excel™, or database programs such as Microsoft Access™ or Paradox™) can be adapted to the present invention by inputting one or more character string corresponding, e.g., to an expression pattern or profile, subject medical or historical data, molecular signature, or the like, into the software which is loaded into the memory of a digital system, and carrying out the operations indicated in an instruction set. For example, systems can include the foregoing software having the appropriate character string information, e.g., used in conjunction with a user interface in conjunction with a standard operating system such as a Windows, Macintosh or LINUX system. For example, an instruction set for manipulating strings of characters, either by programming the required operations into the applications or with the required operations performed manually by a user (or both). For example, specialized sequence alignment programs such as PILEUP or BLAST can also be incorporated into the systems of the invention, e.g., for alignment of nucleic acids or proteins (or corresponding character strings).

Software for performing the statistical methods required for the invention, e.g., to determine correlations between expression profiles and subsets of members of the diagnostic nucleotide libraries, such as programmed embodiments of the statistical methods described above, are also included in the computer systems of the invention. Alternatively, programming elements for performing such methods as principle component analysis (PCA) or least squares analysis can also be included in the digital system to identify relationships between data. Exemplary software for such methods is provided by Partek, Inc., St. Peter, Mo; at the web site partek.com.

Any controller or computer optionally includes a monitor which can include, e.g., a flat panel display (e.g., active matrix liquid crystal display, liquid crystal display), a cathode ray tube ("CRT") display, or another display system which serves as a user interface, e.g., to output predictive data. Computer circuitry, including numerous integrated circuit chips, such as a microprocessor, memory, interface circuits, and the like, is often placed in a casing or box which optionally also includes a hard disk drive, a floppy disk drive, a high capacity removable drive such as a writeable CD-ROM, and other common peripheral elements.

Inputting devices such as a keyboard, mouse, or touch sensitive screen, optionally provide for input from a user and for user selection, e.g., of sequences or data sets to be compared or otherwise manipulated in the relevant computer system. The computer typically includes appropriate software for receiving user instructions, either in the form of user input into a set parameter or data fields (e.g., to input relevant subject data), or in the form of preprogrammed instructions, e.g., preprogrammed for a variety of different specific operations. The software then converts these instructions to appropriate language for instructing the system to carry out any desired operation.

The integrated system may also be embodied within the circuitry of an application specific integrated circuit (ASIC) or programmable logic device (PLD). In such a case, the invention is embodied in a computer readable descriptor language that can be used to create an ASIC or PLD. The integrated system can also be embodied within the circuitry or logic processors of a variety of other digital apparatus, such as PDAs, laptop computer systems, displays, image editing equipment, etc.

The digital system can comprise a learning component where expression profiles, and relevant subject data are compiled and monitored in conjunction with physical assays, and where correlations, e.g., molecular signatures with predictive value for a disease, are established or refined. Successful and unsuccessful combinations are optionally documented in a database to provide justification/preferences for user-base or digital system based selection of diagnostic nucleotide sets with high predictive accuracy for a specified disease or condition.

The integrated systems can also include an automated workstation. For example, such a workstation can prepare and analyze leukocyte RNA samples by performing a sequence of events including: preparing RNA from a human blood sample; labeling the RNA with an isotopic or non-isotopic label; hybridizing the labeled RNA to at least one array comprising all or part of the candidate library; and detecting the hybridization pattern. The hybridization pattern is digitized and recorded in the appropriate database.

Automated RNA preparation tool

The invention also includes an automated RNA preparation tool for the preparation of mononuclear cells from whole blood samples, and preparation of RNA from the mononuclear cells. In a preferred embodiment, the use of the RNA preparation tool is fully automated, so that the cell separation and RNA isolation would require no human manipulations. Full automation is advantageous because it minimizes delay, and standardizes sample preparation across different laboratories. This standardization increases the reproducibility of the results.

The processes performed by the RNA preparation tool of the invention are as follows. A primary component of the device is a centrifuge. Tubes of whole blood containing a density gradient solution, transcription/translation inhibitors, and a gel barrier that separates erythrocytes from mononuclear cells and serum after centrifugation are placed in the centrifuge. The barrier is permeable to erythrocytes and granulocytes during centrifugation, but does not allow mononuclear cells to pass through (or the barrier substance has a density such that mononuclear cells remain above the level of the barrier during the centrifugation). After centrifugation, the erythrocytes and granulocytes are trapped beneath the barrier, facilitating isolation of the mononuclear cell and serum layers. A mechanical arm removes the tube and inverts it to mix the mononuclear cell layer and the serum. The arm next pours the supernatant into a fresh tube, while the erythrocytes and granulocytes remained below the barrier. Alternatively, a needle is used to aspirate the supernatant and transfer it to a fresh tube. The mechanical arms of the device opens and closes lids, dispenses PBS to aid in the collection of the mononuclear cells by centrifugation, and moves the tubes in and out of the centrifuge. Following centrifugation, the supernatant is poured off or removed by a vacuum device, leaving an isolated mononuclear cell pellet. Purification of the RNA from the cells is performed automatically, with lysis buffer and other purification solutions automatically dispensed and removed before and after centrifugation steps. The result is a purified RNA solution. In another embodiment, RNA isolation is performed using a column or filter method. In yet another embodiment, the invention includes an on-board homogenizer for use in cell lysis.

Other automated systems

Automated and/or semi-automated methods for solid and liquid phase high-throughput sample preparation and evaluation are available, and supported by commercially available devices. For example, robotic devices for preparation of nucleic acids from bacterial colonies, e.g., to facilitate production and characterization of the candidate library include, for example, an automated colony picker (e.g., the Q-bot, Genetix, U.K.) capable of identifying, sampling, and inoculating up to 10,000/4 hrs different clones into 96 well microtiter dishes. Alternatively, or in addition, robotic systems for liquid handling are available from a variety of sources, e.g., automated workstations like the automated synthesis apparatus developed by Takeda Chemical Industries, LTD. (Osaka, Japan) and many robotic systems utilizing robotic arms (Zymate II, Zymark Corporation, Hopkinton, Mass.; Orca, Beckman Coulter, Inc. (Fullerton, CA)) which mimic the manual operations performed by a scientist. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput analysis of library components or subject leukocyte samples. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

High throughput screening systems that automate entire procedures, e.g., sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the relevant assay are commercially available. (see, e.g., Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, etc.). These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, arrays and array readers are available, e.g., from Affymetrix, PE Biosystems, and others.

The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like.

A variety of commercially available peripheral equipment, including, e.g., optical and fluorescent detectors, optical and fluorescent microscopes, plate readers, CCD arrays, phosphorimagers, scintillation counters, phototubes, photodiodes, and the like, and software is available for digitizing, storing and analyzing a digitized video or digitized optical or other assay results, e.g., using PC (Intel x86 or pentium chip- compatible DOS™, OS2™ WINDOWS™, WINDOWS NT™ or WINDOWS95™ based machines), MACINTOSH™, or UNIX based (e.g., SUN™ work station) computers.

Embodiment in a web site.

The methods described above can be implemented in a localized or distributed computing environment. For example, if a localized computing environment is used, an array comprising a candidate nucleotide library, or diagnostic nucleotide set, is configured in proximity to a detector, which is, in turn, linked to a computational device equipped with user input and output features.

In a distributed environment, the methods can be implemented on a single computer with multiple processors or, alternatively, on multiple computers. The computers can be linked, e.g. through a shared bus, but more commonly, the computer(s) are nodes on a network. The network can be

generalized or dedicated, at a local level or distributed over a wide geographic area. In certain embodiments, the computers are components of an intra-net or an internet.

The predictive data corresponding to subject molecular signatures (e.g., expression profiles, and related diagnostic, prognostic, or monitoring results) can be shared by a variety of parties. In particular, such information can be utilized by the subject, the subject's health care practitioner or provider, a company or other institution, or a scientist. An individual subject's data, a subset of the database or the entire database recorded in a computer readable medium can be accessed directly by a user by any method of communication, including, but not limited to, the internet. With appropriate computational devices, integrated systems, communications networks, users at remote locations, as well as users located in proximity to, e.g., at the same physical facility, the database can access the recorded information. Optionally, access to the database can be controlled using unique alphanumeric passwords that provide access to a subset of the data. Such provisions can be used, e.g., to ensure privacy, anonymity, etc.

Typically, a client (e.g., a patient, practitioner, provider, scientist, or the like) executes a Web browser and is linked to a server computer executing a Web server. The Web browser is, for example, a program such as IBM's Web Explorer, Internet explorer, NetScape or Mosaic, or the like. The Web server is typically, but not necessarily, a program such as IBM's HTTP Daemon or other WWW daemon (e.g., LINUX-based forms of the program). The client computer is bi-directionally coupled with the server computer over a line or via a wireless system. In turn, the server computer is bi-directionally coupled with a website (server hosting the website) providing access to software implementing the methods of this invention.

A user of a client connected to the Intranet or Internet may cause the client to request resources that are part of the web site(s) hosting the application(s) providing an implementation of the methods described herein. Server program(s) then process the request to return the specified resources (assuming they are currently available). A standard naming convention has been adopted, known as a Uniform Resource Locator ("URL"). This convention encompasses several types of location names, presently including subclasses such as Hypertext Transport Protocol ("http"), File Transport Protocol ("ftp"), gopher, and Wide Area Information Service ("WAIS"). When a resource is downloaded, it may include the URLs of additional resources. Thus, the user of the client can easily learn of the existence of new resources that he or she had not specifically requested.

Methods of implementing Intranet and/or Intranet embodiments of computational and/or data access processes are well known to those of skill in the art and are documented, e.g., in ACM Press, pp. 383-392; ISO-ANSI, Working Draft, "Information Technology-Database Language SQL", Jim Melton, Editor, International Organization for Standardization and American National Standards Institute, Jul. 1992; ISO Working Draft, "Database Language SQL-Part 2:Foundation (SQL/Foundation)", CD9075-2:199.chi.SQL, Sep. 11, 1997; and Cluer et al. (1992) A General Framework for the Optimization of Object-Oriented Queries, Proc SIGMOD International Conference on Management of Data, San Diego, California, Jun. 2-5, 1992, SIGMOD Record, vol. 21, Issue 2, Jun., 1992; Stonebraker, M., Editor;. Other resources are available, e.g., from Microsoft, IBM, Sun and other software development companies.

Using the tools described above, users of the reagents, methods and database as discovery or diagnostic tools can query a centrally located database with expression and subject data. Each submission of data adds to the sum of expression and subject information in the database. As data is added, a new correlation statistical analysis is automatically run that incorporates the added clinical and expression data. Accordingly, the predictive accuracy and the types of correlations of the recorded molecular signatures increases as the database grows.

For example, subjects, such as patients, can access the results of the expression analysis of their leukocyte samples and any accrued knowledge regarding the likelihood of the patient's belonging to any specified diagnostic (or prognostic, or monitoring, or risk group), i.e., their expression profiles, and/or molecular signatures. Optionally, subjects can add to the predictive accuracy of the database by providing additional information to the database regarding diagnoses, test results, clinical or other related events that have occurred since the time of the expression profiling. Such information can be provided to the database via any form of communication, including, but not limited to, the internet. Such data can be used to continually define (and redefine) diagnostic groups. For example, if 1000 patients submit data regarding the occurrence of myocardial infarction over the 5 years since their expression profiling, and 300 of these patients report that they have experienced a myocardial infarction and 700 report that they have not, then the 300 patients define a new "group A." As the algorithm is used to continually query and revise the database, a new diagnostic nucleotide set that differentiates groups A and B (i.e., with and without myocardial infarction within a five year period) is identified. This newly defined nucleotide set is then be used (in the manner described above) as a test that predicts the occurrence of myocardial infarction over a five-year period. While submission directly by the patient is exemplified above, any individual with access and authority to submit the relevant data e.g., the patient's physician, a laboratory technician, a health care or study administrator, or the like, can do so.

As will be apparent from the above examples, transmission of information via the internet (or via an intranet) is optionally bi-directional. That is, for example, data regarding expression profiles, subject data, and the like are transmitted via a communication system to the database, while information regarding molecular signatures, predictive analysis, and the like, are transmitted from the database to the user. For example, using appropriate configurations of an integrated system including a microarray comprising a diagnostic nucleotide set, a detector linked to a computational device can directly transmit (locally or from a remote workstation at great distance, e.g., hundreds or thousands of miles distant from the database) expression profiles and a corresponding individual identifier to a central database for analysis according to the methods of the invention. According to, e.g., the algorithms described above, the individual identifier is assigned to one or more diagnostic (or prognostic, or monitoring, etc.) categories. The results of this classification are then relayed back, via, e.g., the same mode of communication, to a recipient at the same or different internet (or intranet) address.

Kits

The present invention is optionally provided to a user as a kit. Typically, a kit contains one or more diagnostic nucleotide sets of the invention. Alternatively, the kit contains the candidate

nucleotide library of the invention. Most often, the kit contains a diagnostic nucleotide probe set, or other subset of a candidate library, (e.g., as a cDNA, oligonucleotide or antibody microarray or reagents for performing an assay on a diagnostic gene set using any expression profiling technology), packaged in a suitable container. The kit may further comprise, one or more additional reagents, e.g., substrates, labels, primers, for labeling expression products, tubes and/or other accessories, reagents for collecting blood samples, buffers, e.g., erythrocyte lysis buffer, leukocyte lysis buffer, hybridization chambers, cover slips, etc., as well as a software package, e.g., including the statistical methods of the invention, e.g., as described above, and a password and/or account number for accessing the compiled database. The kit optionally further comprises an instruction set or user manual detailing preferred methods of using the diagnostic nucleotide sets in the methods of the invention. In one embodiment, the kit may include contents useful for the discovery of diagnostic nucleotide sets using microarrays. The kit may include sterile, endotoxin and RNAse free blood collection tubes. The kit may also include alcohol swabs, tourniquet, blood collection set, and/or PBS (phosphate buffer saline; needed when method of example 8 is used to derived mononuclear RNA). The kit may also include cell lysis buffer. The kit may include RNA isolation kit, substrates for labeling of RNA (may vary for various expression profiling techniques). The kit may also include materials for fluorescence microarray expression profiling, including one or more of the following: reverse transcriptase and 10x RT buffer, T7(dT)24 primer (primer with T7 promoter at 5' end), DTT, deoxynucleotides, optionally 100mM each, RNAse inhibitor, second strand cDNA buffer, DNA polymerase, Rnase H, T7 RNA polymerase ribonucleotides, in vitro transcription buffer, and/or Cy3 and Cy5 labeled ribonucleotides. The kit may also include microarrays containing candidate gene libraries, cover slips for slides, and/or hybridization chambers. The kit may further include software package for identification of diagnostic gene set from data, that contains statistical methods, and/or allows alteration in desired sensitivity and specificity of gene set. The software may further facilitate access to and data analysis by centrally a located database server. The software may further include a password and account number to access central database server. In addition, the kit may include a kit user manual.

In another embodiment, the kit may include contents useful for the application of diagnostic nucleotide sets using microarrays. The kit may include sterile, endotoxin and/or RNAse free blood collection tubes. The kit may also include, alcohol swabs, tourniquet, and/or a blood collection set. The kit may further include PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA), cell lysis buffer, and/or an RNA isolation kit. In addition, the kit may include substrates for labeling of RNA (may vary for various expression profiling techniques). For fluorescence microarray expression profiling, components may include reverse transcriptase and 10x RT buffer, T7(dT)24 primer (primer with T7 promoter at 5' end), DTT, deoxynucleotides (optionally 100mM each), RNAse inhibitor, second strand cDNA buffer, DNA polymerase, Rnase H, T7 RNA polymerase, ribonucleotides, in vitro transcription buffer, and/or Cy3 and Cy5 labeled ribonucleotides. The kit may further include microarrays containing candidate gene libraries. The kit may also include cover slips for slides, and/or hybridization chambers. The kit may include a software package for identification of diagnostic gene set from data. The software package may contain statistical methods, allow alteration in desired sensitivity and specificity of gene set, and/or facilitate access to and data

analysis by centrally located database server. The software package may include a password and account number to access central database server. In addition, the kit may include a kit user manual.

In another embodiment, the kit may include contents useful for the application of diagnostic nucleotide sets using real-time PCR. This kit may include terile, endotoxin and/or RNAse free blood collection tubes. The kit may further include alcohol swabs, tourniquet, and/or a blood collection set. The kit may also include PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA). In addition, the kit may include cell lysis buffer and/or an RNA isolation kit. The kit may laso include substrates for real time RT-PCR, which may vary for various real-time PCR techniques, including poly dT primers, random hexamer primers, reverse Transcriptase and RT buffer, DTT, deoxynucleotides 100 mM, RNase H, primer pairs for diagnostic and control gene set, 10x PCR reaction buffer, and/or Taq DNA polymerase. The kit may also include fluorescent probes for diagnostic and control gene set (alternatively, fluorescent dye that binds to only double stranded DNA). The kit may further include reaction tubes with or without barcode for sample tracking, 96-well plates with barcode for sample identification, one barcode for entire set, or individual barcode per reaction tube in plate. The kit may also include a software package for identification of diagnostic gene set from data, and /or statistical methods. The software package may allow alteration in desired sensitivity and specificity of gene set, and/or facilitate access to and data analysis by centrally located database server. The kit may include a password and account number to access central database server. Finally, the kit may include a kit user manual.

This invention will be better understood by reference to the following non-limiting Examples:

Examples:

Example 1: Preparation of RNA from mononuclear cells for expression profiling
Example 2: Preparation of Universal Control RNA for use in leukocyte expression profiling
Example 3: Identification of diagnostic oligonucleotide sets for use in diagnosis of rheumatoid arthritis.
Example 4: Identification of diagnostic oligonucleotide sets for diagnosis of Systemic Lupus Erythematosis
Example 5: Design of oligonucleotide probes.
Example 6: Production of an array of 8,000 spotted 50 mer oligonucleotides.
Example 7: Amplification, labeling, and hybridization of total RNA to an oligonucleotide microarray
Example 8: Real-time PCR validation of array expression results
Example 9: Correlation and Classification Analysis
Example 10: Assay sample preparation
Example 11: Identification and validation of gene expression markers for diagnosis and monitoring of lupus and autoimmune diseases.

Examples

Example 1: Preparation of RNA from mononuclear cells for expression profiling

Blood was isolated from the subject for leukocyte expression profiling using the following methods:

Two tubes were drawn per patient. Blood was drawn from either a standard peripheral venous blood draw or directly from a large-bore intra-arterial or intravenous catheter inserted in the femoral artery, femoral vein, subclavian vein or internal jugular vein. Care was taken to avoid sample contamination with heparin from the intravascular catheters, as heparin can interfere with subsequent RNA reactions.

For each tube, 8 ml of whole blood was drawn into a tube (CPT, Becton-Dickinson order #362753) containing the anticoagulant Citrate, 25°C density gradient solution (e.g. Ficoll, Percoll) and a polyester gel barrier that upon centrifugation was permeable to RBCs and granulocytes but not to mononuclear cells. The tube was inverted several times to mix the blood with the anticoagulant. The tubes were centrifuged at 1750xg in a swing-out rotor at room temperature for 20 minutes. The tubes were removed from the centrifuge and inverted 5-10 times to mix the plasma with the mononuclear cells, while trapping the RBCs and the granulocytes beneath the gel barrier. The plasma/mononuclear cell mix was decanted into a 15ml tube and 5ml of phosphate-buffered saline (PBS) is added. The 15ml tubes were spun for 5 minutes at 1750xg to pellet the cells. The supernatant was discarded and 1.8 ml of RLT lysis buffer is added to the mononuclear cell pellet. The buffer and cells were pipetted up and down to ensure complete lysis of the pellet. The cell lysate was frozen and stored until it is convenient to proceed with isolation of total RNA.

Total RNA was purified from the lysed mononuclear cells using the Qiagen Rneasy Miniprep kit, as directed by the manufacturer (10/99 version) for total RNA isolation, including homogenization (Qiashredder columns) and on-column DNase treatment. The purified RNA was eluted in 50ul of water.

Some samples were prepared by a different protocol, as follows:

Two 8 ml blood samples were drawn from a peripheral vein into a tube (CPT, Becton-Dickinson order #362753) containing anticoagulant (Citrate), 25°C density gradient solution (Ficoll) and a polyester gel barrier that upon centrifugation is permeable to RBCs and granulocytes but not to mononuclear cells. The mononuclear cells and plasma remained above the barrier while the RBCs and granulocytes were trapped below. The tube was inverted several times to mix the blood with the anticoagulant, and the tubes were subjected to centrifugation at 1750xg in a swing-out rotor at room temperature for 20 min. The tubes were removed from the centrifuge, and the clear plasma layer above the cloudy mononuclear cell layer was aspirated and discarded. The cloudy mononuclear cell layer was aspirated, with care taken to rinse all of the mononuclear cells from the surface of the gel barrier with PBS (phosphate buffered saline). Approximately 2 mls of mononuclear cell suspension was transferred to a 2ml microcentrifuge tube, and centrifuged for 3min. at 16,000 rpm in a microcentrifuge to pellet the cells. The supernatant was discarded and 1.8 ml of RLT lysis buffer (Qiagen) were added to the mononuclear cell pellet, which lysed the cells and inactivated Rnases. The cells and lysis buffer were

pipetted up and down to ensure complete lysis of the pellet. Cell lysate was frozen and stored until it was convenient to proceed with isolation of total RNA.

RNA samples were isolated from 8 mL of whole blood. Yields ranged from 2 μg to 20μg total RNA for 8mL blood. A260/A280 spectrophotometric ratios were between 1.6 and 2.0, indicating purity of sample. 2ul of each sample were run on an agarose gel in the presence of ethidium bromide. No degradation of the RNA sample and no DNA contamination were visible.

In some cases, specific subsets of mononuclear cells were isolated from peripheral blood of human subjects. When this was done, the StemSep cell separation kits (manual version 6.0.0) were used from StemCell Technologies (Vancouver, Canada). This same protocol can be applied to the isolation of T cells, CD4 T cells, CD8 T cells, B cells, monocytes, NK cells and other cells. Isolation of cell types using negative selection with antibodies may be desirable to avoid activation of target cells by antibodies.

Example 2: Preparation of Universal Control RNA for use in leukocyte expression profiling

Control RNA was prepared using total RNA from Buffy coats and/or total RNA from enriched mononuclear cells isolated from Buffy coats, both with and without stimulation with ionomycin and PMA. The following control RNAs were prepared:

Control 1: Buffy Coat Total RNA
Control 2: Mononuclear cell Total RNA
Control 3: Stimulated buffy coat Total RNA
Control 4: Stimulated mononuclear Total RNA
Control 5: 50% Buffy coat Total RNA / 50% Stimulated buffy coat Total RNA
Control 6: 50% Mononuclear cell Total RNA / 50% Stimulated Mononuclear Total RNA.

Some samples were prepared using the following protocol: Buffy coats from 38 individuals were obtained from Stanford Blood Center. Each buffy coat is derived from ~350 mL whole blood from one individual. 10 ml buffy coat was removed from the bag, and placed into a 50 ml tube. 40 ml of Buffer EL (Qiagen) was added, the tube was mixed and placed on ice for 15 minutes, then cells were pelleted by centrifugation at 2000xg for 10 minutes at 4°C. The supernatant was decanted and the cell pellet was re-suspended in 10 ml of Qiagen Buffer EL. The tube was then centrifuged at 2000xg for 10 minutes at 4°C. The cell pellet was then re-suspended in 20 ml TRIZOL (GibcoBRL) per Buffy coat sample, the mixture was shredded using a rotary homogenizer, and the lysate was then frozen at –80°C prior to proceeding to RNA isolation.

Other control RNAs were prepared from enriched mononuclear cells prepared from Buffy coats. Buffy coats from Stanford Blood Center were obtained, as described above. 10 ml buffy coat was added to a 50 ml polypropylene tube, and 10 ml of phosphate buffer saline (PBS) was added to each tube. A polysucrose (5.7 g/dL) and sodium diatrizoate (9.0 g/dL) solution at a 1.077 +/-0.0001 g/ml density solution of equal volume to diluted sample was prepared (Histopaque 1077, Sigma cat. no 1077-1). This and all subsequent steps were performed at room temperature. 15 ml of diluted buffy coat/PBS was layered on top of 15 ml of the histopaque solution in a 50 ml tube. The tube was centrifuged at 400xg for 30 minutes at room temperature. After centrifugation, the upper layer of the solution to within 0.5 cm of the opaque interface containing the mononuclear cells was discarded. The

opaque interface was transferred into a clean centrifuge tube. An equal volume of PBS was added to each tube and centrifuged at 350xg for 10 minutes at room temperature. The supernatant was discarded. 5 ml of Buffer EL (Qiagen) was used to resuspend the remaining cell pellet and the tube was centrifuged at 2000xg for 10 minutes at room temperature. The supernatant was discarded. The pellet was resuspended in 20 ml of TRIZOL (GibcoBRL) for each individual buffy coat that was processed. The sample was homogenized using a rotary homogenizer and frozen at -80C until RNA was isolated.

RNA was isolated from frozen lysed Buffy coat samples as follows: frozen samples were thawed, and 4 ml of chloroform was added to each buffy coat sample. The sample was mixed by vortexing and centrifuged at 2000xg for 5 minutes. The aqueous layer was moved to new tube and then repurified by using the RNeasy Maxi RNA clean up kit, according to the manufacturer's instruction (Qiagen, PN 75162). The yield, purity and integrity were assessed by spectrophotometer and gel electrophoresis.

Some samples were prepared by a different protocol, as follows. The further use of RNA prepared using this protocol is described in Example 7.

50 whole blood samples were randomly selected from consented blood donors at the Stanford Medical School Blood Center. Each buffy coat sample was produced from ~350 mL of an individual's donated blood. The whole blood sample was centrifuged at ~4,400 x g for 8 minutes at room temperature, resulting in three distinct layers: a top layer of plasma, a second layer of buffy coat, and a third layer of red blood cells. 25 ml of the buffy coat fraction was obtained and diluted with an equal volume of PBS (phosphate buffered saline). 30 ml of diluted buffy coat was layered onto 15 ml of sodium diatrizoate solution adjusted to a density of 1.077+/-0.001 g/ml (Histopaque 1077, Sigma) in a 50mL plastic tube. The tube was spun at 800 g for 10 minutes at room temperature. The plasma layer was removed to the 30 ml mark on the tube, and the mononuclear cell layer removed into a new tube and washed with an equal volume of PBS, and collected by centrifugation at 2000 g for 10 minutes at room temperature. The cell pellet was resuspended in 10 ml of Buffer EL (Qiagen) by vortexing and incubated on ice for 10 minutes to remove any remaining erthythrocytes. The mononuclear cells were spun at 2000 g for 10 minutes at 4 degrees Celsius. The cell pellet was lysed in 25 ml of a phenol/guanidinium thiocyanate solution (TRIZOL Reagent, Invitrogen). The sample was homogenized using a PowerGene 5 rotary homogenizer (Fisher Scientific) and Omini disposable generator probes (Fisher Scientific). The Trizol lysate was frozen at –80 degrees C until the next step.

The samples were thawed out and incubated at room temperature for 5 minutes. 5 ml chloroform was added to each sample, mixed by vortexing, and incubated at room temperature for 3 minutes. The aqueous layers were transferred to new 50 ml tubes. The aqueous layer containing total RNA was further purified using the Qiagen RNeasy Maxi kit (PN 75162), per the manufacturer's protocol (October 1999). The columns were eluted twice with 1 ml Rnase-free water, with a minute incubation before each spin. Quantity and quality of RNA was assessed using standard methods. Generally, RNA was isolated from batches of 10 buffy coats at a time, with an average yield per buffy coat of 870 μg, and an estimated total yield of 43.5 mg total RNA with a 260/280 ratio of 1.56 and a 28S/18S ratio of 1.78.

Quality of the RNA was tested using the Agilent 2100 Bioanalyzer using RNA 6000 microfluidics chips. Analysis of the electrophorgrams from the Bioanalyzer for five different batches demonstrated the reproducibility in quality between the batches.

Total RNA from all five batches were combined and mixed in a 50 ml tube, then aliquoted as follows: 2 x 10 ml aliquots in 15 ml tubes, and the rest in 100 µl aliquots in 1.5 ml microcentrifuge tubes. The aliquots gave highly reproducible results with respect to RNA purity, size and integrity. The RNA was stored at –80°C.

Test hybridization of Reference RNA.

When compared with BC38 and Stimulated mononuclear reference samples, the R50 performed as well, if not better than the other reference samples as shown in Figure 4.

In an analysis of hybridizations, where the R50 targets were fluorescently labeled with Cy-5 using methods described herein and the amplified and labeled aRNA was hybridized (as in example 7) to the olignoucleotide array described in example 6. The R50 detected **97.3%** of probes with a Signal to Noise ratio (S/N) of greater than three and **99.9 %** of probes with S/N greater one.

Example 3: Identification of diagnostic oligonucleotides and oligonucleotide sets for use in monitoring treatment and/or progression of Rheumatoid arthritis

Rheumatoid arthritis (hereinafter, "RA") is a chronic and debilitating inflammatory arthritis. The diagnosis of RA is made by clinical criteria and radiographs. A new class of medication, TNF blockers, are effective, but the drugs are expensive, have side effects and not all patients respond to treatment. In addition, relief of disease symptoms does not always correlate with inhibition of joint destruction. For these reasons, an alternative mechanism for the titration of therapy is needed.

An observational study was conducted in which a cohort of patients meeting American College of Rheumatology (hereinafter "ARC") criteria for the diagnosis of RA was identified. Arnett et al. (1988) Arthritis Rheum 31:315-24. Patients gave informed consent and a peripheral blood mononuclear cell RNA sample was obtained by the methods as described herein. When available, RNA samples were also obtained from surgical specimens of bone or synovium from effected joints, and synovial fluid. Also, T-cells were isolated from the peripheral blood for some patients for expression analysis. This was done using the protocol given in Example 1.

From each patient, the following clinical information was obtained if available: Demographic information; information relating to the ACR criteria for RA; presence or absence of additional diagnoses of inflammatory and non-inflammatory conditions; data from laboratory test, including complete blood counts with differentials, CRP, ESR, ANA, Serum IL6, Soluble CD40 ligand, LDL, HDL, Anti-DNA antibodies, rheumatoid factor, C3, C4, serum creatinine and any medication levels; data from surgical procedures such as gross operative findings and pathological evaluation of resected tissues and biopsies; information on pharmacological therapy and treatment changes; clinical diagnoses of disease "flare"; hospitalizations; quantitative joint exams; results from health assessment questionnaires (HAQs); other clinical measures of patient symptoms and disability; physical examination results and radiographic data assessing joint involvement, synovial thickening, bone loss and erosion and joint space narrowing and deformity. In some cases, data includes pathological evaluation of synovial memebranes and joint tissues from RA and control patients. Pathology scoring

systems were used to determine disease category, inflammation, type of inflammatory infiltrate, cellular and makeup of the synovial inflammation.

For some specimens of synovium, mononuclear cells or subsets of mononuclear cells (such as T cells) can be isolated for expression profiling. The relative number of lyphocyte subsets for some specimens can be determined by fluorescence activated cell sorting. Examples are determination of the CD4/CD8 T-cell ratio for a specimen. This information can be used as a variable to correlate to other outcomes or as an outcome for correlation analysis.

From these data, measures of improvement in RA are derived as exemplified by the ACR 20% and 50% response/improvement rates (Felson et al. 1996). Measures of disease activity over some period of time is derived from these data as are measures of disease progression. Serial radiography of effected joints is used for objective determination of progression (e.g., joint space narrowing, peri-articular osteoporosis, synovial thickening). Disease activity is determined from the clinical scores, medical history, physical exam, lab studies, surgical and pathological findings.

The collected clinical data (disease criteria) is used to define patient or sample groups for correlation of expression data. Patient groups are identified for comparison, for example, a patient group that possesses a useful or interesting clinical distinction, verses a patient group that does not possess the distinction. Examples of useful and interesting patient distinctions that can be made on the basis of collected clinical data are listed here:

Samples from patients during a clinically diagnosed RA flare versus samples from these same or different patients while they are asymptomatic.

Samples from patients who subsequently have high measures of disease activity versus samples from those same or different patients who have low subsequent disease activity.

Samples from patients who subsequently have high measures of disease progression versus samples from those same or different patients who have low subsequent disease progression.

Samples from patients who subsequently respond to a given medication or treatment regimen versus samples from those same or different patients who subsequently do not respond to a given medication or treatment regimen (for example, TNF pathway blocking medications).

Samples from patients with a diagnosis of osteoarthritis versus patients with rheumatoid arthritis.

Samples from patients with tissue biopsy results showing a high degree of inflammation versus samples from patients with lesser degrees of histological evidence of inflammation on biopsy.

Expression profiles correlating with progression of RA are identified. Subsets of the candidate library (or a previously identified diagnostic nucleotide set) are identified, according to the above procedures, that have predictive value for the progression of RA.

Diagnostic nucleotide set(s) are identified which predict respond to TNF blockade. Patients are profiled before and during treatment with these medications. Patients are followed for relief of symptoms, side effects and progression of joint destruction, e.g., as measured by hand radiographs. Expression profiles correlating with response to TNF blockade are identified. Subsets of the candidate library (or a previously identified diagnostic nucleotide set) are identified, according to the above procedures that have predictive value for response to TNF blockade.

Example 4: Identification of diagnostic oligonucleotide and oligonucleotide sets for diagnosis of Systemic Lupus Erythematosis

SLE is a chronic, systemic inflammatory disease characterized by dysregulation of the immune system. Clinical manifestations affect every organ system and include skin rash, renal dysfunction, CNS disorders, arthralgias and hematologic abnormalities. SLE clinical manifestations tend to both recur intermittently (or "flare") and progress over time, leading to permanent end-organ damage.

An observational study was conducted in which a cohort of patients meeting American College of Rheumatology (hereinafter "ACR") criteria for the diagnosis of SLE were identified. See Tan et al. (1982) Arthritis Rheum 25:1271-7. Patients gave informed consent and a peripheral blood mononuclear cell RNA sample or a peripheral T cell sample was obtained by the methods as described in example 1.

From each patient, the following clinical information was obtained if available: Demographic information, ACR criteria for SLE, additional diagnoses of inflammatory and non-inflammatory conditions, data from laboratory testing including complete blood counts with differentials, CRP, ESR, ANA, Serum IL6, Soluble CD40 ligand, LDL, HDL, Anti-DNA antibodies, rheumatoid factor, C3, C4, serum creatinine (and other measures of renal dysfunction), medication levels, data from surgical procedures such as gross operative findings and pathological evaluation of resected tissues and biopsies (e.g., renal, CNS), information on pharmacological therapy and treatment changes, clinical diagnoses of disease "flare", hospitalizations, quantitative joint exams, results from health assessment questionnaires (HAQs), SLEDAIs (a clinical score for SLE activity that assess many clinical variables; Bombadier C, Gladman DD, Urowitz MB, Caron D, Chang CH and the Committee on Prognosis Studies in SLE: Derivation of the SLEDAI for Lupus Patients. Arthritis Rheum 35:630-640, 1992), other clinical measures of patient symptoms and disability, physical examination results and carotid ultrasonography.

The collected clinical data (disease criteria) is used to define patient or sample groups for correlation of expression data. Patient groups are identified for comparison, for example, a patient group that possesses a useful or interesting clinical distinction, verses a patient group that does not possess the distinction. Measures of disease activity in SLE are derived from the clinical data described above to divide patients (and patient samples) into groups with higher and lower disease activity over some period of time or at any one point in time. Such data are SLEDAI scores and other clinical scores, levels of inflammatory markers or complement, number of hospitalizations, medication use and changes, biopsy results and data measuring progression of end-organ damage or end-organ damage, including progressive renal failure, carotid atherosclerosis, and CNS dysfunction.

Expression profiles correlating with progression of SLE are identified, including expression profiles corresponding to end-organ damage and progression of end-organ damage. Expression profiles are identified predicting disease progression or disease "flare", response to treatment or likelihood of response to treatment, predict likelihood of "low" or "high" disease measures (optionally described using the SLEDAI score), and presence or likelihood of developing premature carotid

atherosclerosis. Subsets of the candidate library (or a previously identified diagnostic nucleotide set) are identified, according to the above procedures that have predictive value for the progression of SLE.

Further examples of useful and interesting patient distinctions that can be made on the basis of collected clinical data are listed here. Samples can be grouped and groups are compared to discover diagnostic gene sets:

1. Samples from patients during a clinically diagnosed SLE flare versus samples from these same or different patients while they are asymptomatic or while they have a documented infection.
2. Samples from patients who subsequently have high measures of disease activity versus samples from those same or different patients who have low subsequent disease activity.
3. Samples from patients who subsequently have high measures of disease progression versus samples from those same or different patients who have low subsequent disease progression.
4. Samples from patients who subsequently respond to a given medication or treatment regimen versus samples from those same or different patients who subsequently do not respond to a given medication or treatment regimen.
5. Samples from patients with premature carotid atherosclerosis on ultrasonography versus patients with SLE without premature atherosclerosis.

Identification of a diagnostic oligonucleotide or oligonucleotide set for diagnosis of lupus

Mononuclear RNA samples were collected from patients with SLE and patients with Rheumatoid or Osteoarthritis (RA and OA) or controls using the protocol described in example 1. The patient diagnoses were determined using standard diagnostic algorithms such as those that are employed by the American College of Rheumatology (see example See Tan et al. (1982) Arthritis Rheum 25:1271-7; Arnett et al. (1988) Arthritis Rheum 31:315-24).

32 samples were included in the anaysis. 15 samples were derived from patients with a clinical diagnosis of SLE and the remainder were derived from patients with RA (9), OA (4) and subjects without known disease (4) who served as controls. Samples from patients with SLE or RA were classified as "Active" or "Controlled" (with respect to disease activity) by the patient's physician based on objective and subjective criteria, such as patient history, physical exam and lab studies. An attempt was made to match SLE patients and controls with respect to important variables such as medication use, sex, age and secondary diagnoses.

After preparation of RNA (example 1), amplification, labeling, hybridization, scanning, feature extraction and data processing were done as described in Example 7 using the oligonucleotide microarrays described in Example 6. The resulting log ratio of expression of Cy3 (patient sample)/ Cy5 (R50 reference RNA) was used for analysis.

Initially, significance analysis for microarrays (SAM, Tusher 2001, Example 9) was used to discover that were differentially expressed between 7 of the Lupus samples and 17 control samples. 1 gene was identified that was expressed at a higher level in the lupus patients than in all controls. This gene had a 0.5% false detection rate using SAM. This means that there is statistically, a 99.5% chance that the gene is truly differentially expressed between the Lupus and control samples. This gene was oligonucleotide and SEQ ID # 518. The oligonucleotide:

GCCTCTTGCTTGGCGTGATAACCCTGTCATCTTCCCAAAGCTCATTTATG detects a specific human gene: sialyltransferase (SIAT4A), Unigene : Hs.301698
Locus: NM_003033, GI: 4506950. Expression ratios for the gene are given for each sample in Figure 5A-B. The average fold change in expression between SLE and controls was 1.48.

When a larger data set was used, 15 SLE samples were compared to 17 controls. Using SAM, genes were identified as significantly differentially expressed between Lupus and controls. Supervised harvesting classification (X-Mine, Brisbane, CA) and CART (Salford Systems, San Diego CA) were also used on the same data to determine which set of genes best distinguish SLE from control samples (Example 9).

CART was used to build a decision tree for classification of samples as lupus or not lupus using the gene expression data from the arrays. The analysis identitifies sets of genes that can be used together to accurately identify samples derived from lupus patients. The set of genes and the identified threshold expression levels for the decision tree are referred to as "models". Multiple models for diagnosis of Lupus were derived by using different settings and parameters for the CART algorithm and using different sets of genes in the analysis. When using CART, it may be desirable to limit the number of independent variables. In the case of the genes on the arrays, a subset of ~8000 can be selected for analysis in CART based on significant differential expression discovered by using SAM or some other algorithm.

Model I was based on a data set consisting of thirty-two samples (fifteen SLE and seventeen non-SLE). These samples were used to derive the model and are referred to a the "training set'. Model I used the expression values for twenty-nine genes, which were found to be most significant in differentiating SLE and non-SLE samples in the analysis using SAM described above. SLE samples were designated as Class 1 and non-SLE samples were designated as Class 2. For this analysis, the following settings were used in the MODEL SETUP (CART, Salford Systems, San Diego, CA.). In the Model settings, the tree type selected for the analysis was classification. In the Categorical settings, the default values were used. In the Testing settings, V-fold cross-validation was selected with a value of 10. In the Select Cases settings, the default values were used. In the Best Tree settings, the default values were used. In the Combine settings, the default values were used. In the Method settings, Symmetric Gini was selected as the type of classification tree and Linear combinations for splitting was also selected. The default values were used for the linear combinations. In the Advance Settings, the default values were used. In the Costs settings, the default values were used. In the Priors settings, Equal was selected as the priors for Class. In the penalty settings, the default values were used.

From this analysis, CART built two models, a two-gene model and a three-gene model (Figures 5C-E). The sensitivity and specificity for the identification of lupus in the training set samples of the two genes model were 100% and 94%, respectively. The sensitivity and specificity for the 10-fold cross validation set of the two-gene model were 100% and 88%, respectively, with a relative cost of 0.118. The sensitivity and specificity for the training set of the three genes model were 100% and 100%, respectively. The sensitivity and specificity for the 10-fold cross validation set of the three genes model were 93% and 94%, respectively, with a relative cost of 0.125.

Model II was based on a data set consisted of thirty-two samples, fifteen SLE and seventeen non-SLE (training set) and six thousand forty-four genes with expression values for at least 80% of the samples. The MODEL SETUP for the analysis of this data set was the same as for the analysis above, except for the following correction. In the Method settings, Linear combination for splitting was unchecked after the analysis yielded no classification tree. The change in the linear combination setting resulted in the following.

The sensitivity and specificity for the training set of the one gene model were 87% and 82%, respectively. The sensitivity and specificity for the 10-fold cross validation set of the one gene model were 80% and 59%, respectively, with a relative cost of 0.612. The sensitivity and specificity for the training set of the three genes model were 100% and 88%, respectively. The sensitivity and specificity for the 10-fold cross validation set of the three genes model were 67% and 65%, respectively, with a relative cost of 0.686. The sensitivity and specificity for the training set of the five genes model were 100% and 94%, respectively. The sensitivity and specificity for the 10-fold cross validation set of the five genes model were 67% and 59%, respectively, with a relative cost of 0.745. Results and models are summarized in Figure 5 C and F.

Those genes that were found to be useful for classification are noted in Table 2.

These genes can be used alone or in association with other genes or variables to build a diagnostic gene set or a classification algorithm. These genes can be used in association with known gene markers for lupus (such as those identified in the prior art) to provide a diagnostic algorithm.

Primers for real-time PCR validation were designed for some of the genes as described in Example 8 and are listed in Table 2B.

Surrogates for some of the most useful genes were identified. Surrogates can be used in addition to or in place of a diagnostic gene in a method of detecting lupus or in diagnostic gene set. For genes that were splitters in CART, surrogates were identified and reported by the software. In these cases, the best available surrogates are listed. For other genes, hierarchical clustering of the data was performed with default settings (x-miner, X-mine, Brisbane, CA) and members of gene expression clusters were noted. A cluster was selected that included the gene of interest and the members of that cluster were recorded in Table 2D.

Example 5- Design of oligonucleotide probes

By way of example, this section describes the design of four oligonucleotide probes using Array Designer Ver 1.1 (Premier Biosoft International, Palo Alto, CA). The major steps in the process are given first.

1) Obtain best possible sequence of mRNA from GenBank. If a full-length sequence reference sequence is not available, a partial sequence is used, with preference for the 3' end over the 5' end. When the sequence is known to represent the antisense strand, the reverse complement of the sequence is used for probe design. For sequences represented in the subtracted leukocyte expression library that have no significant match in GenBank at the time of probe design, our sequence is used.

2) Mask low complexity regions and repetitive elements in the sequence using an algorithm such as RepeatMasker.

3) Use probe design software, such as Array Designer, version 1.1, to select a sequence of 50 residues with specified physical and chemical properties. The 50 residues nearest the 3' end constitute a search frame. The residues it contains are tested for suitability. If they don't meet the specified criteria, the search frame is moved one residue closer to the 5' end, and the 50 residues it now contains are tested. The process is repeated until a suitable 50-mer is found.

4) If no such 50-mer occurs in the sequence, the physical and chemical criteria are adjusted until a suitable 50-mer is found.

5) Compare the probe to dbEST, the UniGene cluster set, and the assembled human genome using the BLASTn search tool at NCBI to obtain the pertinent identifying information and to verify that the probe does not have significant similarity to more than one known gene.

Clone 40H12

Clone 40H12 was sequenced and compared to the nr, dbEST, and UniGene databases at NCBI using the BLAST search tool. The sequence matched accession number NM_002310, a 'curated RefSeq project' sequence, see Pruitt et al. (2000) Trends Genet. 16:44-47, encoding leukemia inhibitory factor receptor (LIFR) mRNA with a reported E value of zero. An E value of zero indicates there is, for all practical purposes, no chance that the similarity was random based on the length of the sequence and the composition and size of the database. This sequence, cataloged by accession number NM_002310, is much longer than the sequence of clone 40H12 and has a poly-A tail. This indicated that the sequence cataloged by accession number NM_002310 is the sense strand and a more complete representation of the mRNA than the sequence of clone 40H12, especially at the 3' end. Accession number "NM_002310" was included in a text file of accession numbers representing sense strand mRNAs, and sequences for the sense strand mRNAs were obtained by uploading a text file containing desired accession numbers as an Entrez search query using the Batch Entrez web interface and saving the results locally as a FASTA file. The following sequence was obtained, and the region of alignment of clone 40H12 is outlined:

CTCTCTCCCAGAACGTGTCTCTGCTGCAAGGCACCGGGCCCTTTCGCTCTGCAGAACTGCACTTGCAAGA
CCATTATCAACTCCTAATCCCAGCTCAGAAAGGGAGCCTCTGCGACTCATTCATCGCCCTCCAGGACTGA
CTGCATTGCACAGATGATGGATATTTACGTATGTTTGAAACGACCATCCTGGATGGTGGACAATAAAAGA
ATGAGGACTGCTTCAAATTTCCAGTGGCTGTTATCAACATTTATTCTTCTATATCTAATGAATCAAGTAA
ATAGCCAGAAAAAGGGGGCTCCTCATGATTTGAAGTGTGTAACTAACAATTTGCAAGTGTGGAACTGTTC
TTGGAAAGCACCCTCTGGAACAGGCCGTGGTACTGATTATGAAGTTTGCATTGAAAACAGGTCCCGTTCT
TGTTATCAGTTGGAGAAAACCAGTATTAAAATTCCAGCTCTTTCACATGGTGATTATGAAATAACAATAA
ATTCTCTACATGATTTTGGAAGTTCTACAAGTAAATTCACACTAAATGAACAAAACGTTTCCTTAATTCC
AGATACTCCAGAGATCTTGAATTTGTCTGCTGATTTCTCAACCTCTACATTATACCTAAAGTGGAACGAC
AGGGGTTCAGTTTTTCCACACCGCTCAAATGTTATCTGGGAAATTAAAGTTCTACGTAAAGAGAGTATGG
AGCTCGTAAAATTAGTGACCCACAACACAACTCTGAATGGCAAAGATACACTTCATCACTGGAGTTGGGC
CTCAGATATGCCCTTGGAATGTGCCATTCATTTTGTGGAAATTAGATGCTACATTGACAATCTTCATTTT
TCTGGTCTCGAAGAGTGGAGTGACTGGAGCCCTGTGAAGAACATTTCTTGGATACCTGATTCTCAGACTA
AGGTTTTTCCTCAAGATAAAGTGATACTTGTAGGCTCAGACATAACATTTTGTTGTGTGAGTCAAGAAAA
AGTGTTATCAGCACTGATTGGCCATACAAACTGCCCCTTGATCCATCTTGATGGGGAAAATGTTGCAATC
AAGATTCGTAATATTTCTGTTTCTGCAAGTAGTGGAACAAATGTAGTTTTTACAACCGAAGATAACATAT
TTGGAACCGTTATTTTTGCTGGATATCCACCAGATACTCCTCAACAACTGAATTGTGAGACACATGATTT
AAAAGAAATTATATGTAGTTGGAATCCAGGAAGGGTGACAGCGTTGGTGGGCCCACGTGCTACAAGCTAC

ACTTTAGTTGAAAGTTTTTCAGGAAAATATGTTAGACTTAAAAGAGCTGAAGCACCTACAAACGAAAGCT
ATCAATTATTATTTCAAATGCTTCCAAATCAAGAAATATATAATTTTACTTTGAATGCTCACAATCCGCT
GGGTCGATCACAATCAACAATTTTAGTTAATATAACTGAAAAAGTTTATCCCCATACTCCTACTTCATTC
AAAGTGAAGGATATTAATTCAACAGCTGTTAAACTTTCTTGGCATTTACCAGGCAACTTTGCAAAGATTA
ATTTTTTATGTGAAATTGAAATTAAGAAATCTAATTCAGTACAAGAGCAGCGGAATGTCACAATCAAAGG
AGTAGAAAATTCAAGTTATCTTGTTGCTCTGGACAAGTTAAATCCATACACTCTATATACTTTTCGGATT
CGTTGTTCTACTGAAACTTTCTGGAAATGGAGCAAATGGAGCAATAAAAAACAACATTTAACAACAGAAG
CCAGTCCTTCAAAGGGGCCTGATACTTGGAGAGAGTGGAGTTCTGATGGAAAAAATTTAATAATCTATTG
GAAGCCTTTACCCATTAATGAAGCTAATGGAAAAATACTTTCCTACAATGTATCGTGTTCATCAGATGAG
GAAACACAGTCCCTTTCTGAAATCCCTGATCCTCAGCACAAAGCAGAGATACGACTTGATAAGAATGACT
ACATCATCAGCGTAGTGGCTAAAAATTCTGTGGGCTCATCACCACCTTCCAAAATAGCGAGTATGGAAAT
TCCAAATGATGATCTCAAAATAGAACAAGTTGTTGGGATGGGAAAGGGGATTCTCCTCACCTGGCATTAC
GACCCCAACATGACTTGCGACTACGTCATTAAGTGGTGTAACTCGTCTCGGTCGGAACCATGCCTTATGG
ACTGGAGAAAAGTTCCCTCAAACAGCACTGAAACTGTAATAGAATCTGATGAGTTTCGACCAGGTATAAG
ATATAATTTTTTCCTGTATGGATGCAGAAATCAAGGATATCAATTATTACGCTCCATGATTGGATATATA
GAAGAATTGGCTCCCATTGTTGCACCAAATTTTACTGTTGAGGATACTTCTGCAGATTCGATATTAGTAA
AATGGGAAGACATTCCTGTGGAAGAACTTAGAGGCTTTTTAAGAGGATATTTGTTTTACTTTGGAAAAGG
AGAAAGAGACACATCTAAGATGAGGGTTTTAGAATCAGGTCGTTCTGACATAAAAGTTAAGAATATTACT
GACATATCCCAGAAGACACTGAGAATTGCTGATCTTCAAGGTAAAACAAGTTACCACCTGGTCTTGCGAG
CCTATACAGATGGTGGAGTGGGCCCGGAGAAGAGTATGTATGTGGTGACAAAGGAAAATTCTGTGGGATT
AATTATTGCCATTCTCATCCCAGTGGCAGTGGCTGTCATTGTTGGAGTGGTGACAAGTATCCTTTGCTAT
CGGAAACGAGAATGGATTAAAGAAACCTTCTACCCTGATATTCCAAATCCAGAAAACTGTAAAGCATTAC
AGTTTCAAAAGAGTGTCTGTGAGGGAAGCAGTGCTCTTAAAACATTGGAAATGAATCCTTGTACCCCAAA
TAATGTTGAGGTTCTGGAAACTCGATCAGCATTTCCTAAAATAGAAGATACAGAAATAATTTCCCCAGTA
GCTGAGCGTCCTGAAGATCGCTCTGATGCAGAGCCTGAAAACCATGTGGTTGTGTCCTATTGTCCACCCA
TCATTGAGGAAGAAATACCAAACCCAGCCGCAGATGAAGCTGGAGGGACTGCACAGGTTATTTACATTGA
TGTTCAGTCGATGTATCAGCCTCAAGCAAAACCAGAAGAAGAACAAGAAAATGACCCTGTAGGAGGGGCA
GGCTATAAGCCACAGATGCACCTCCCCATTAATTCTACTGTGGAAGATATAGCTGCAGAAGAGGACTTAG
ATAAAACTGCGGGTTACAGACCTCAGGCCAATGTAAATACATGGAATTTAGTGTCTCCAGACTCTCCTAG
ATCCATAGACAGCAACAGTGAGATTGTCTCATTTGGAAGTCCATGCTCCATTAATTCCCGACAATTTTTG
ATTCCTCCTAAAGATGAAGACTCTCCTAAATCTAATGGAGGAGGGTGGTCCTTTACAAACTTTTTTCAGA
ACAAACCAAACGATTAACAGTGTCACCGTGTCACTTCAGTCAGCCATCTCAATAAGCTCTTACTGCTAGT
GTTGCTACATCAGCACTGGGCATTCTTGGAGGGATCCTGTGAAGTATTGTTAGGAGGTGAACTTCACTAC
ATGTTAAGTTACACTGAAAGTTCATGTGCTTTTAATGTAGTCTAAAAGCCAAAGTATAGTGACTCAGAAT
CCTCAATCCACAAAACTCAAGATTGGGAGCTCTTTGTGATCAAGCCAAAGAATTCTCATGTACTCTACCT
TCAAGAAGCATTTCAAGGCTAATACCTACTTGTACGTACATGTAAAACAAATCCCGCCGCAACTGTTTTC
TGTTCTGTTGTTTGTGGTTTTCTCATATGTATACTTGGTGGAATTGTAAGTGGATTTGCAGGCCAGGGAG
AAAATGTCCAAGTAACAGGTGAAGTTTATTTGCCTGACGTTTACTCCTTTCTAGATGAAAACCAAGCACA
GATTTTAAAACTTCTAAGATTATTCTCCTCTATCCACAGCATTCACAAAAATTAATATAATTTTTAATGT
AGTGACAGCGATTTAGTGTTTTGTTTGATAAAGTATGCTTATTTCTGTGCCTACTGTATAATGGTTATCA
AACAGTTGTCTCAGGGGTACAAACTTTGAAAACAAGTGTGACACTGACCAGCCCAAATCATAATCATGTT
TTCTTGCTGTGATAGGTTTTGCTTGCCTTTTCATTATTTTTAGCTTTTATGCTTGCTTCCATTATTTCA
GTTGGTTGCCCTAATATTTAAAATTTACACTTCTAAGACTAGAGACCCACATTTTTTAAAAATCATTTTA
TTTTGTGATACAGTGACAGCTTTATATGAGCAAATTCAATATTATTCATAAGCATGTAATTCCAGTGACT
TACTATGTGAGATGACTACTAAGCAATATCTAGCAGCGTTAGTTCCATATAGTTCTGATTGGATTTCGTT
CCTCCTGAGGAGACCATGCCGTTGAGCTTGGCTACCCAGGCAGTGGTGATCTTTGACACCTTCTGGTGGA
TGTTCCTCCCACTCATGAGTCTTTTCATCATGCCACATTATCTGATCCAGTCCTCACATTTTTAAATATA
AAACTAAAGAGAGAATGCTTCTTACAGGAACAGTTACCCAAGGGCTGTTTCTTAGTAACTGTCATAAACT
GATCTGGATCCATGGGCATACCTGTGTTCGAGGTGCAGCAATTGCTTGGTGAGCTGTGCAGAATTGATTG
CCTTCAGCACAGCATCCTCTGCCCACCCTTGTTTCTCATAAGCGATGTCTGGAGTGATTGTGGTTCTTGG
AAAAGCAGAAGGAAAAACTAAAAAGTGTATCTTGTATTTTCCCTGCCCTCAGGTTGCCTATGTATTTTAC
CTTTTCATATTTAAGGCAAAAGTACTTGAAAATTTTAAGTGTCCGAATAAGATATGTCTTTTTTGTTTGT
TTTTTTTGGTTGGTTGTTTGTTTTTTATCATCTGAGATTCTGTAATGTATTTGCAAATAATGGATCAATT
AATTTTTTTTGAAGCTCATATTGTATCTTTTTAAAAACCATGTTGTGGAAAAAAGCCAGAGTGACAAGTG
ACAAAATCTATTTAGGAACTCTGTGTATGAATCCTGATTTTAACTGCTAGGATTCAGCTAAATTTCTGAG
CTTTATGATCTGTGGAAATTTGGAATGAAATCGAATTCATTTTGTACATACATAGTATATTAAAACTATA
TAATAGTTCATAGAAATGTTCAGTAATGAAAAAATATATCCAATCAGAGCCATCCCGAAAAAAAAAAAAA
AA (SEQ ID NO:1039)

The FASTA file, including the sequence of NM_002310, was masked using the RepeatMasker web interface (Smit, AFA & Green, P RepeatMasker at genome.washington.edu/RM/RepeatMasker.html, Smit and Green). Specifically, during masking, the following types of sequences were replaced with "N's": SINE/MIR & LINE/L2, LINE/L1 , LTR/MaLR, LTR/Retroviral , Alu, and other low informational content sequences such as simple repeats. Below is the sequence following masking:

CTCTCTCCCAGAACGTGTCTCTGCTGCAAGGCACCGGGCCCTTTCGCTCTGCAGAACTGCACTTGCAAG
ACCATTATCAACTCCTAATCCCAGCTCAGAAAGGGAGCCTCTGCGACTCATTCATCGCCCTCCAGGACT
GACTGCATTGCACAGATGATGGATATTTACGTATGTTTGAAACGACCATCCTGGATGGTGGACAATAAA
AGAATGAGGACTGCTTCAAATTTCCAGTGGCTGTTATCAACATTTATTCTTCTATATCTAATGAATCAA
GTAAATAGCCAGAAAAAGGGGGCTCCTCATGATTTGAAGTGTGTAACTAACAATTTGCAAGTGTGGAAC
TGTTCTTGGAAAGCACCCTCTGGAACAGGCCGTGGTACTGATTATGAAGTTTGCATTGAAAACAGGTCC
CGTTCTTGTTATCAGTTGGAGAAAACCAGTATTAAAATTCCAGCTCTTTCACATGGTGATTATGAAATA
ACAATAAATTCTCTACATGATTTTGGAAGTTCTACAAGTAAATTCACACTAAATGAACAAAACGTTTCC
TTAATTCCAGATACTCCAGAGATCTTGAATTTGTCTGCTGATTTCTCAACCTCTACATTATACCTAAAG
TGGAACGACAGGGGTTCAGTTTTTCCACACCGCTCAAATGTTATCTGGGAAATTAAAGTTCTACGTAAA
GAGAGTATGGAGCTCGTAAAATTAGTGACCCACAACACAACTCTGAATGGCAAAGATACACTTCATCAC
TGGAGTTGGGCCTCAGATATGCCCTTGGAATGTGCCATTCATTTTGTGGAAATTAGATGCTACATTGAC
AATCTTCATTTTTCTGGTCTCGAAGAGTGGAGTGACTGGAGCCCTGTGAAGAACATTTCTTGGATACCT
GATTCTCAGACTAAGGTTTTTCCTCAAGATAAAGTGATACTTGTAGGCTCAGACATAACATTTTGTTGT
GTGAGTCAAGAAAAAGTGTTATCAGCACTGATTGGCCATACAAACTGCCCCTTGATCCATCTTGATGGG
GAAAATGTTGCAATCAAGATTCGTAATATTTCTGTTTCTGCAAGTAGTGGAACAAATGTAGTTTTTACA
ACCGAAGATAACATATTTGGAACCGTTATTTTTGCTGGATATCCACCAGATACTCCTCAACAACTGAAT
TGTGAGACACATGATTTAAAAGAAATTATATGTAGTTGGAATCCAGGAAGGGTGACAGCGTTGGTGGGC
CCACGTGCTACAAGCTACACTTTAGTTGAAAGTTTTTCAGGAAAATATGTTAGACTTAAAAGAGCTGAA
GCACCTACAAACGAAAGCTATCAATTATTATTTCAAATGCTTCCAAATCAAGAAATATATAATTTTACT
TTGAATGCTCACAATCCGCTGGGTCGATCACAATCAACAATTTTAGTTAATATAACTGAAAAAGTTTAT
CCCCATACTCCTACTTCATTCAAAGTGAAGGATATTAATTCAACAGCTGTTAAACTTTCTTGGCATTTA
CCAGGCAACTTTGCAAAGATTAATTTTTTATGTGAAATTGAAATTAAGAAATCTAATTCAGTACAAGAG
CAGCGGAATGTCACAATCAAAGGAGTAGAAAATTCAAGTTATCTTGTTGCTCTGGACAAGTTAAATCCA
TACACTCTATATACTTTTCGGATTCGTTGTTCTACTGAAACTTTCTGGAAATGGAGCAAATGGAGCAAT
AAAAAACAACATTTAACAACAGAAGCCAGTCCTTCAAAGGGGCCTGATACTTGGAGAGAGTGGAGTTCT
GATGGAAAAAATTTAATAATCTATTGGAAGCCTTTACCCATTAATGAAGCTAATGGAAAAATACTTTCC
TACAATGTATCGTGTTCATCAGATGAGGAAACACAGTCCCTTTCTGAAATCCCTGATCCTCAGCACAAA
GCAGAGATACGACTTGATAAGAATGACTACATCATCAGCGTAGTGGCTAAAAATTCTGTGGGCTCATCA
CCACCTTCCAAAATAGCGAGTATGGAAATTCCAAATGATGATCTCAAAATAGAACAAGTTGTTGGGATG
GGAAAGGGGATTCTCCTCACCTGGCATTACGACCCCAACATGACTTGCGACTACGTCATTAAGTGGTGT
AACTCGTCTCGGTCGGAACCATGCCTTATGGACTGGAGAAAAGTTCCCTCAAACAGCACTGAAACTGTA
ATAGAATCTGATGAGTTTCGACCAGGTATAAGATATAATTTTTTCCTGTATGGATGCAGAAATCAAGGA
TATCAATTATTACGCTCCATGATTGGATATATAGAAGAATTGGCTCCCATTGTTGCACCAAATTTTACT

GTTGAGGATACTTCTGCAGATTCGATATTAGTAAAATGGGAAGACATTCCTGTGGAAGAACTTAGAGGC
TTTTTAAGAGGATATTTGTTTTACTTTGGAAAAGGAGAAAGAGACACATCTAAGATGAGGGTTTTAGAA
TCAGGTCGTTCTGACATAAAAGTTAAGAATATTACTGACATATCCCAGAAGACACTGAGAATTGCTGAT
CTTCAAGGTAAAACAAGTTACCACCTGGTCTTGCGAGCCTATACAGATGGTGGAGTGGGCCCGGAGAAG
AGTATGTATGTGGTGACAAAGGAAAATTCTGTGGGATTAATTATTGCCATTCTCATCCCAGTGGCAGTG
GCTGTCATTGTTGGAGTGGTGACAAGTATCCTTTGCTATCGGAAACGAGAATGGATTAAAGAAACCTTC
TACCCTGATATTCCAAATCCAGAAAACTGTAAAGCATTACAGTTTCAAAAGAGTGTCTGTGAGGGAAGC
AGTGCTCTTAAAACATTGGAAATGAATCCTTGTACCCCAAATAATGTTGAGGTTCTGGAAACTCGATCA
GCATTTCCTAAAATAGAAGATACAGAAATAATTTCCCCAGTAGCTGAGCGTCCTGAAGATCGCTCTGAT
GCAGAGCCTGAAAACCATGTGGTTGTGTCCTATTGTCCACCCATCATTGAGGAAGAAATACCAAACCCA
GCCGCAGATGAAGCTGGAGGGACTGCACAGGTTATTTACATTGATGTTCAGTCGATGTATCAGCCTCAA
GCAAAACCAGAAGAAGAACAAGAAAATGACCCTGTAGGAGGGGCAGGCTATAAGCCACAGATGCACCTC
CCCATTAATTCTACTGTGGAAGATATAGCTGCAGAAGAGGACTTAGATAAAACTGCGGGTTACAGACCT
CAGGCCAATGTAAATACATGGAATTTAGTGTCTCCAGACTCTCCTAGATCCATAGACAGCAACAGTGAG
ATTGTCTCATTTGGAAGTCCATGCTCCATTAATTCCCGACAATTTTTGATTCCTCCTAAAGATGAAGAC
TCTCCTAAATCTAATGGAGGAGGGTGGTCCTTTACAAACTTTTTTCAGAACAAACCAAACGATTAACAG
TGTCACCGTGTCACTTCAGTCAGCCATCTCAATAAGCTCTTACTGCTAGTGTTGCTACATCAGCACTGG
GCATTCTTGGAGGGATCCTGTGAAGTATTGTTAGGAGGTGAACTTCACTACATGTTAAGTTACACTGAA
AGTTCATGTGCTTTTAATGTAGTCTAAAAGCCAAAGTATAGTGACTCAGAATCCTCAATCCACAAAACT
CAAGATTGGGAGCTCTTTGTGATCAAGCCAAAGAATTCTCATGTACTCTACCTTCAAGAAGCATTTCAA
GGCTAATACCTACTTGTACGTACATGTAAAACAAATCCCGCCGCAACTGTTTTCTGTTCTGTTGTTTGT
GGTTTTCTCATATGTATACTTGGTGGAATTGTAAGTGGATTTGCAGGCCAGGGAGAAAATGTCCAAGTA
ACAGGTGAAGTTTATTTGCCTGACGTTTACTCCTTTCTAGATGAAAACCAAGCACAGATTTTAAAACTT
CTAAGATTATTCTCCTCTATCCACAGCATTCACNNNNNNNNNNNNNNNNNNNNNNNGTAGTGACAGCGAT
TTAGTGTTTTGTTTGATAAAGTATGCTTATTTCTGTGCCTACTGTATAATGGTTATCAAACAGTTGTCT
CAGGGGTACAAACTTTGAAAACAAGTGTGACACTGACCAGCCCAAATCATAATCATGTTTTCTTGCTGT
GATAGGTTTTGCTTGCCTTTTCATTATTTTTTAGCTTTTATGCTTGCTTCCATTATTTCAGTTGGTTGC
CCTAATATTTAAAATTTACACTTCTAAGACTAGAGACCCACATTTTTTAAAAATCATTTTATTTTGTGA
TACAGTGACAGCTTTATATGAGCAAATTCAATATTATTCATAAGCATGTAATTCCAGTGACTTACTATG
TGAGATGACTACTAAGCAATATCTAGCAGCGTTAGTTCCATATAGTTCTGATTGGATTTCGTTCCTCCT
GAGGAGACCATGCCGTTGAGCTTGGCTACCCAGGCAGTGGTGATCTTTGACACCTTCTGGTGGATGTTC
CTCCCACTCATGAGTCTTTTCATCATGCCACATTATCTGATCCAGTCCTCACATTTTTAAATATAAAAC
TAAAGAGAGAATGCTTCTTACAGGAACAGTTACCCAAGGGCTGTTTCTTAGTAACTGTCATAAACTGAT
CTGGATCCATGGGCATACCTGTGTTCGAGGTGCAGCAATTGCTTGGTGAGCTGTGCAGAATTGATTGCC
TTCAGCACAGCATCCTCTGCCCACCCTTGTTTCTCATAAGCGATGTCTGGAGTGATTGTGGTTCTTGGA
AAAGCAGAAGGAAAAACTAAAAAGTGTATCTTGTATTTTCCCTGCCCTCAGGTTGCCTATGTATTTTAC
CTTTTCATATTTAAGGCAAAAGTACTTGAAAATTTTAAGTGTCCGAATAAGATATGTCTTTTTTGTTTG
TTTTTTTTGGTTGGTTGTTTGTTTTTTATCATCTGAGATTCTGTAATGTATTTGCAAATAATGGATCAA
TTAATTTTTTTTGAAGCTCATATTGTATCTTTTTAAAAACCATGTTGTGGAAAAAAGCCAGAGTGACAA
GTGACAAAATCTATTTAGGAACTCTGTGTATGAATCCTGATTTTAACTGCTAGGATTCAGCTAAATTTC

TGAGCTTTATGATCTGTGGAAATTTGGAATGAAATCGAATTCATTTTGTACATACATAGTATATTAAAA
CTATATAATAGTTCATAGAAATGTTCAGTAATGAAAAAATATATCCAATCAGAGCCATCCCGAAAAAAA
AAAAAAAA (SEQ ID NO:1040).

The length of this sequence was determined using batch, automated computational methods and the sequence, as sense strand, its length, and the desired location of the probe sequence near the 3' end of the mRNA was submitted to Array Designer Ver 1.1 (Premier Biosoft International, Palo Alto, CA). Search quality was set at 100%, number of best probes set at 1, length range set at 50 base pairs, Target Tm set at 75 C. degrees plus or minus 5 degrees, Hairpin max deltaG at 6.0 -kcal/mol., Self dimmer max deltaG at 6.0 –kcal/mol, Run/repeat (dinucleotide) max length set at 5, and Probe site minimum overlap set at 1. When none of the 49 possible probes met the criteria, the probe site would be moved 50 base pairs closer to the 5' end of the sequence and resubmitted to Array Designer for analysis. When no possible probes met the criteria, the variation on melting temperature was raised to plus and minus 8 degrees and the number of identical basepairs in a run increased to 6 so that a probe sequence was produced.

In the sequence above, using the criteria noted above, Array Designer Ver 1.1 designed a probe with the following sequence oligonucleotide SEQ ID NO:1041 and is indicated by underlining in the sequence above. It has a melting temperature of 68.4 degrees Celsius and a max run of 6 nucleotides and represents one of the cases where the criteria for probe design in Array Designer Ver 1.1 were relaxed in order to obtain an oligonucleotide near the 3' end of the mRNA (Low melting temperature was allowed).

Clone 463D12

Clone 463D12 was sequenced and compared to the nr, dbEST, and UniGene databases at NCBI using the BLAST search tool. The sequence matched accession number AI184553, an EST sequence with the definition line "qd60a05.x1 Soares_testis_NHT Homo sapiens cDNA clone IMAGE:1733840 3' similar to gb:M29550 PROTEIN PHOSPHATASE 2B CATALYTIC SUBUNIT 1 (HUMAN);, mRNA sequence." The E value of the alignment was 1.00×10^{-118}. The GenBank sequence begins with a poly-T region, suggesting that it is the antisense strand, read 5' to 3'. The beginning of this sequence is complementary to the 3' end of the mRNA sense strand. The accession number for this sequence was included in a text file of accession numbers representing antisense sequences. Sequences for antisense strand mRNAs were obtained by uploading a text file containing desired accession numbers as an Entrez search query using the Batch Entrez web interface and saving the results locally as a FASTA file. The following sequence was obtained, and the region of alignment of clone 463D12 is outlined:

TTTTTTTTTTTTTTCTTAAATAGCATTTATTTTCTCTCAAAAAGCCTATTATGTACTAACAAGTGTTCC
TCTAAATTAGAAAGGCATCACTACTAAAATTTTATACATATTTTTTATATAAGAGAAGGAATATTGGGT
TACAATCTGAATTTCTCTTTATGATTTCTCTTAAAGTATAGAACAGCTATTAAAATGACTAATATTGCT
AAAATGAAGGCTACTAAATTTCCCCAAGAATTTCGGTGGAATGCCCAAAAATGGTGTTAAGATATGCAG

AAGGGCCCATTTCAAGCAAAGCAATCTCTCCACCCCTTCATAAAAGATTTAAGCTAAAAAAAAAAAAAA

AAGAAGAAAATCCAACAGCTGAAGACATTGGGCTATTTATAAATCTTCTCCCAGTCCCCCAGACAGCCT

CACATGGGGGCTGTAAACAGCTAACTAAAATATCTTTGAGACTCTTATGTCCACACCCACTGACACAAG

GAGAGCTGTAACCACAGTGAAACTAGACTTTGCTTTCCTTTAGCAAGTATGTGCCTATGATAGTAAACT

GGAGTAAATGTAACAGTAATAAAACAAATTTTTTTAAAAATAAAAATTATACCTTTTTCTCCAACAAA

CGGTAAAGACCACGTGAAGACATCCATAAAATTAGGCAACCAGTAAAGATGTGGAGAACCAGTAAACTG

TCGAAATTCATCACATTATTTTCATACTTTAATACAGCAGCTTTAATTATTGGAGAACATCAAAGTAAT

TAGGTGCCGAAAAACATTGTTATTAATGAAGGGAACCCCTGACGTTTGACCTTTTCTGTACCATCTATA

GCCCTGGACTTGA (SEQ ID NO: 1042)

The FASTA file, including the sequence of AA184553, was then masked using the RepeatMasker web interface, as shown below. The region of alignment of clone 463D12 is outlined.

TTTTTTTTTTTTTTCTTAAATAGCATTTATTTTCTCTCAAAAAGCCTATTATGTACTAACAAGTGTTCC

TCTAAATTAGAAAGGCATCACTACNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGAGAAGGAATATTGGGT

TACAATCTGAATTTCTCTTTATGATTTCTCTTAAAGTATAGAACAGCTATTAAAATGACTAATATTGCT

AAAATGAAGGCTACTAAATTTCCCCAAGAATTTCGGTGGAATGCCCAAAAATGGTGTTAAGATATGCAG

AAGGGCCCATTTCAAGCAAAGCAATCTCTCCACCCCTTCATAAAAGATTTAAGCTAAAAAAAAAAAAAA

AAGAAGAAAATCCAACAGCTGAAGACATTGGGCTATTTATAAATCTTCTCCCAGTCCCCCAGACAGCCT

CACATGGGGGCTGTAAACAGCTAACTAAAATATCTTTGAGACTCTTATGTCCACACCCACTGACACAAG

GAGAGCTGTAACCACAGTGAAACTAGACTTTGCTTTCCTTTAGCAAGTATGTGCCTATGATAGTAAACT

GGAGTAAATGTAACAGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCTTTTTCTCCAACAAA

CGGTAAAGACCACGTGAAGACATCCATAAAATTAGGCAACCAGTAAAGATGTGGAGAACCAGTAAACTG

TCGAAATTCATCACATTATTTTCATACTTTAATACAGCAGCTTTAATTATTGGAGAACATCAAAGTAAT

TAGGTGCCGAAAAACATTGTTATTAATGAAGGGAACCCCTGACGTTTGACCTTTTCTGTACCATCTATA

GCCCTGGACTTGA Masked version of 463D12 sequence. (SEQ ID NO: 1043)

The sequence was submitted to Array Designer as described above, however, the desired location of the probe was indicated at base pair 50 and if no probe met the criteria, moved in the 3' direction. The complementary sequence from Array Designer was used, because the original sequence was antisense. The oligonucleotide designed by Array Designer has the following sequence oligonucleotide SEQ ID NO:1044 and is complementary to the underlined sequence above. The probe has a melting temperature of 72.7 degrees centigrade and a max run of 4 nucleotides.

Clone 72D4

Clone 72D4 was sequenced and compared to the nr, dbEST, and UniGene databases at NCBI using the BLAST search tool. No significant matches were found in any of these databases. When compared to the human genome draft, significant alignments were found to three consecutive regions of the reference sequence NT_008060, as depicted below, suggesting that the insert contains three spliced exons of an unidentified gene.

Residue numbers on Matching residue

clone 72D4 sequence	numbers on NT_008060
1 – 198	478646 – 478843
197 – 489	479876 – 480168
491 – 585	489271 – 489365

Because the reference sequence contains introns and may represent either the coding or noncoding strand for this gene, BioCardia's own sequence file was used to design the oligonucleotide. Two complementary probes were designed to ensure that the sense strand was represented. The sequence of the insert in clone 72D4 is shown below, with the three putative exons outlined.

CAGGTCACACAGCACATCAGTGGCTACATGTGAGCTCAGACCTGGGTCTG
CTGCTGTCTGTCTTCCCAATATCCATGACCTTGACTGATGCAGGTGTCTAG
GGATACGTCCATCCCCGTCCTGCTGGAGCCCAGAGCACGGAAGCCTGGCC
CTCCGAGGAGACAGAAGGGAGTGTCGGACACCATGACGAGAGCTTGGCA
GAATAAATAACTTCTTTAAACAATTTTACGGCATGAAGAAATCTGGACCA
GTTTATTAAATGGGATTTCTGCCACAAACCTTGGAAGAATCACATCATCTT
ANNCCCAAGTGAAAACTGTGTTGCGTAACAAAGAACATGACTGCGCTCCA
CACATACATCATTGCCCGGCGAGGCGGACACAAGTCAACGACGGAACA
CTTGAGACAGGCCTACAACTGTGCACGGGTCAGAAGCAAGTTTAAGCCAT
ACTTGCTGCAGTGAGACTACATTTCTGTCTATAGAAGATACCTGACTTGA
TCTGTTTTTCAGCTCCAGTTCCCAGATGTGCGTGTTGTGGTCCCCAAGTAT
CACCTTCCAATTTCTGGGAGCAGTGCTCTGGCCGGATCCTTGCCGCGCGG
ATAAAAAC (SEQ ID NO: 1045)

The sequence was submitted to RepeatMasker, but no repetitive sequences were found. The sequence shown above was used to design the two 50-mer probes using Array Designer as described above. The probes are shown in bold typeface in the sequence depicted below. SEQ ID NO: 1046 and SEQ ID NO:1047

CAGGTCACACAGCACATCAGTGGCTACATGTGAGCTCAGACCTGGGTCTGCTGCTGT
CTGTCTTCCCAATATCCATGACCTTGACTGATGCAGGTGTCTAGGGATACGTCCATC
CCCGTCCTGCTGGAGCCCAGAGCACGGAAGCCTGGCCCTCCGAGGAGACAGAAGGGA
GTGTCGGACACCATGACGAGAGCTTGGCAGAATAAATAACTTCTTTAAACAATTTTA
CGGCATGAAGAAATCTGGACCAGTTTATTAAATGGGATTTCTGCCACAAACCTTGGA
AGAATCACATCATCTTANNCCCAAGTGAAAACTGTGTTGCGTAACAAAGAACATGAC

TGCGCTCCACACATACATCATTGCCCGGCGAGGCGGGACACAAGTCAACGACGGAAC

ACTTGAGACAGGCCTACAACTGTGCACGGGTCAGAAGCAAGTTTAAGCCATACTTGC

TGCAGTGAGACTACATTTCTGTCTATAGAAGATACCTGACTTGATCTGTTTTTCAGC

TC**CAGTTCCCAGATGTGC**

←----

GTCAAGGGTCTACACG

GTGTTGTGGTCCCCAAGTATCACCTTCCAATTTCTGGGAG--→

CACAACACCAGGGGTTCATAGTGGAAGGTTAAAG-5'

CAGTGCTCTGGCCGGATCCTTGCCGCGCGGATAAAAACT---→

Confirmation of probe sequence

Following probe design, each probe sequence was confirmed by comparing the sequence against dbEST, the UniGene cluster set, and the assembled human genome using BLASTn at NCBI. Alignments, accession numbers, gi numbers, UniGene cluster numbers and names were examined and the most common sequence used for the probe. The final probe set was compiled into Table 2. In this table, the sequence ID is given which corresponds to the sequence listing. The origin of the sequence for inclusion on the array is noted as coming from one of the cDNA libraries described in example 1, mining from databases as described in examples 2 and 11 or identification from the published literature. The unigene number, genebank accession and GI number are also given for each sequence when known. The name of the gene associated with the accession number is noted. Finally, the nucleotide sequence of each probe is also given.

Example 6 - Production of an array of 8000 spotted 50mer oligonucleotides

We produced an array of 8000 spotted 50mer oligonucleotides. Examples 11 and 12 exemplify the design and selection of probes for this array.

Sigma-Genosys (The Woodlands, TX) synthesized un-modified 50-mer oligonucleotides using standard phosphoramidite chemistry, with a starting scale of synthesis of 0.05 μmole (see, e.g., R. Meyers, ed. (1995) Molecular Biology and Biotechnology: A Comprehensive Desk Reference). Briefly, to begin synthesis, a 3' hydroxyl nucleoside with a dimethoxytrityl (DMT) group at the 5' end was attached to a solid support. The DMT group was removed with trichloroacetic acid (TCA) in order to free the 5'-hydroxyl for the coupling reaction. Next, tetrazole and a phosphoramidite derivative of the next nucleotide were added. The tetrazole protonates the nitrogen of the phosphoramidite, making it susceptible to nucleophilic attack. The DMT group at the 5'-end of the hydroxyl group blocks further addition of nucleotides in excess. Next, the inter-nucleotide linkage was converted to a phosphotriester bond in an oxidation step using an oxidizing agent and water as the oxygen donor. Excess nucleotides were filtered out and the cycle for the next nucleotide was started by the removal of the DMT protecting group. Following the synthesis, the oligo was cleaved from the solid support. The oligonucleotides were desalted, resuspended in water at a concentration of 100 or 200 μM, and placed

in 96-deep well format. The oligonucleotides were re-arrayed into Whatman Uniplate 384-well polyproylene V bottom plates. The oligonucleotides were diluted to a final concentration 30 μM in 1X Micro Spotting Solution Plus (Telechem/arrayit.com, Sunnyvale, CA) in a total volume of 15 μl. In total, 8,031 oligonucleotides were arrayed into twenty-one 384-well plates.

Arrays were produced on Telechem/arrayit.com Super amine glass substrates (Telechem/arrayit.com), which were manufactured in 0.1 mm filtered clean room with exact dimensions of 25x76x0.96 mm. The arrays were printed using the Virtek Chipwriter with a Telechem 48 pin Micro Spotting Printhead. The Printhead was loaded with 48 Stealth SMP3B TeleChem Micro Spotting Pins, which were used to print oligonucleotides onto the slide with the spot size being 110-115 microns in diameter.

Example 7- Amplification, labeling, and hybridization of total RNA to an oligonucleotide microarray

Amplification, labeling, hybridization and scanning

Samples consisting of at least 0.5 to 2 μg of intact total RNA were further processed for array hybridization. When available, 2 μg of intact total RNA is used for amplification. Amplification and labeling of total RNA samples was performed in three successive enzymatic reactions. First, a single-stranded DNA copy of the RNA was made (hereinafter, "ss-cDNA"). Second, the ss-cDNA was used as a template for the complementary DNA strand, producing double-stranded cDNA (hereinafter, "ds-cDNA, or cDNA"). Third, linear amplification was performed by in vitro transcription from a bacterial T_7 promoter. During this step, fluorescent-conjugated nucleotides were incorporated into the amplified RNA (hereinafter, "aRNA").

The first strand cDNA was produced using the Invitrogen kit (Superscript II). The first strand cDNA was produced in a reaction composed of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, and 3 mM $MgCl_2$ (1x First Strand Buffer, Invitrogen), 0.5 mM dGTP, 0.5 mM dATP, 0.5 mM dTTP, 0.5 mM dCTP, 10 mM DTT, 200 U reverse transcriptase (Superscript II, Invitrogen, #18064014), 15 U RNase inhibitor (RNAGuard, Amersham Pharmacia, #27-0815-01), 5 μM T7T24 primer (5′-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGGTTTTTTTTTTTTTTTTTTTTT-3′), (SEQ ID NO:1048) and 0.5 to 2 μg of selected sample total RNA. Several purified, recombinant control mRNAs from the plant Arabidopsis thaliana were added to the reaction mixture: 2-20 pg of the following genes CAB, RCA, LTP4, NAC1, RCP1,XCP2, RBCL, LTP6, TIM, and PRKase (Stratagene, #252201, #252202, #252204, #252208, #252207, #252206, #252203, #252205, #252209, #252210 respectively). The control RNAs allow the estimate of copy numbers for individual mRNAs in the clinical sample because corresponding sense oligonucleotide probes for each of these plant genes are present on the microarray. The final reaction volume of 20 μl was incubated at 42°C for 90 min.

For synthesis of the second cDNA strand, DNA polymerase and RNase were added to the previous reaction, bringing the final volume to 150 μl. The previous contents were diluted and new substrates were added to a final concentration of 20 mM Tris-HCl (pH 7.0) (Fisher Scientific, Pittsburgh, PA #BP1756-100), 90 mMKCl (Teknova, Half Moon Bay, CA, #0313-500) , 4.6 mM $MgCl_2$ (Teknova, Half Moon Bay, CA, #0304-500), 10 mM$(NH_4)_2SO_4$ (Fisher Scientific #A702-500)(1x Second Strand buffer, Invitrogen), 0.266 mM dGTP, 0.266 mM dATP, 0.266 mM dTTP, 0.266

mM dCTP, 40 U *E. coli* DNA polymerase (Invitrogen, #18010-025), and 2 U RNaseH (Invitrogen, #18021-014). The second strand synthesis took place at 16°C for 150 minutes.

Following second-strand synthesis, the ds-cDNA was purified from the enzymes, dNTPs, and buffers before proceeding to amplification, using phenol-chloroform extraction followed by ethanol precipitation of the cDNA in the presence of glycogen.

Alternatively, a silica-gel column is used to purify the cDNA (e.g. Qiaquick PCR cleanup from Qiagen, #28104). The volume of the column purified cDNA was reduced by ethanol precipitation in the presence of glycogen in which the cDNA was collected by centrifugation at >10,000 ×g for 30 minutes, the supernatant is aspirated, and 150 µl of 70% ethanol, 30% water was added to wash the DNA pellet. Following centrifugation, the supernatant was removed, and residual ethanol was evaporated at room temperature. Alternatively, the volume of the column purified cDNA is reduce in a vacuum evaporator where the supernatant is reduce to a final volume of 7.4 µl.

Linear amplification of the cDNA was performed by in vitro transcription of the cDNA. The cDNA pellet from the step described above was resuspended in 7.4 µl of water, and in vitro transcription reaction buffer was added to a final volume of 20 µl containing 7.5 mM GTP, 7.5 mM ATP, 7.5 mM TTP, 2.25 mM CTP, 1.025 mM Cy3-conjugated CTP (Perkin Elmer; Boston, MA, #NEL-580), 1x reaction buffer (Ambion, Megascript Kit, Austin, TX and #1334) and 1 % T_7 polymerase enzyme mix (Ambion, Megascript Kit, Austin, TX and #1334). This reaction was incubated at 37°C overnight. Following in vitro transcription, the RNA was purified from the enzyme, buffers, and excess NTPs using the RNeasy kit from Qiagen (Valencia, CA; # 74106) as described in the vendor's protocol. A second elution step was performed and the two eluates were combined for a final volume of 60 µl. RNA is quantified using an Agilent 2100 bioanalyzer with the RNA 6000 nano LabChip.

Reference RNA was prepared as described above, except Cy5-CTP was incorporated instead of Cy3CTP. Reference RNA from five reactions, each reaction started with 2 µg total RNA, was pooled together and quantitated as described above.

<u>Hybridization to an array</u>

RNA was prepared for hybridization as follows: for an 18mm×55mm array, 20 µg of amplified RNA (aRNA) was combined with 20 µg of reference aRNA. The combined sample and reference aRNA was concentrated by evaporating the water to 10 µl in a vacuum evaporator. The sample was fragmented by heating the sample at 95°C for 30 minutes to fragment the RNA into 50-200 bp pieces. Alternatively, the combined sample and reference aRNA was concentrated by evaporating the water to 5 µl in a vacuum evaporator. Five µl of 20 mM zinc acetate was added to the aRNA and the mix incubated at 60°C for 10 minutes. Following fragmentation, 40 µl of hybridization buffer was added to achieve final concentrations of 5×SSC and 0.20 %SDS with 0.1 µg/ul of Cot-1 DNA (Invitrogen) as a competitor DNA. The final hybridization mix was heated to 98°C, and then reduced to 50°C at 0.1°C per second.

Alternatively, formamide is included in the hybridization mixture to lower the hybridization temperature.

The hybridization mixture was applied to a pre-heated 65°C microarray, surface, covered with a glass coverslip (Corning, #2935-246), and placed on a pre-heated 65°C hybridization chamber (Telechem, AHC-10). 15 ul of 5xSSC was placed in each of the reservoir in the hybridization chamber and the chamber was sealed and placed in a water bath at 62°C for overnight (16-20 hrs). Following incubation, the slides were washed in 2×SSC, 0.1% SDS for five minutes at 30°C, then in 2×SSC for five minutes at 30°C, then in 2xSSC for another five minutes at 30°C, then in 0.2×SSC for two minutes at room temperature. The arrays were spun at 1000×g for 2 minutes to dry them. The dry microarrays are then scanned by methods described above.

The microarrays were imaged on the Agilent (Palo Alto, CA) scanner G2565AA. The scan settings using the Agilent software were as follows: for the PMT Sensitivity (100% Red and 100% Green); Scan Resolution (10 microns); red and green dye channels; used the default scan region for all slides in the carousel; using the largest scan region; scan date for Instrument ID; and barcode for Slide ID. The full image produced by the Agilent scanner was flipped, rotated, and split into two images (one for each signal channel) using TIFFSplitter (Agilent, Palo Alto, CA). The two channels are the output at 532 nm (Cy3-labeled sample) and 633 nm (Cy5-labeled R50). The individual images were loaded into GenePix 3.0 (Axon Instruments, Union City, CA) for feature extraction, each image was assigned an excitation wavelength corresponding the file opened; Red equals 633 nm and Green equals 532 nm. The setting file (gal) was opened and the grid was laid onto the image so that each spot in the grid overlaped with >50% of the feature. Then the GenePix software was used to find the features without setting minimum threshold value for a feature. For features with low signal intensity, GenePix reports "not found". For all features, the diameter setting was adjusted to include only the feature if necessary.

The GenePix software determined the median pixel intensity for each feature (F_i) and the median pixel intensity of the local background for each feature (B_i) in both channels. The standard deviation (SDF_i and SDB_i) for each is also determined. Features for which GenePix could not discriminate the feature from the background were "flagged" as described below.

Following feature extraction into a " .gpr" file, the header information of the .gpr file was changed to carry accurate information into the database. An Excel macro was written to include the following information: Name of the original .tif image file, SlideID, Version of the feature extraction software, GenePix Array List file, GenePix Settings file, ScanID, Name of person who scanned the slide, Green PMT setting, Red PMT setting, ExtractID (date .gpr file was created, formatted as yyyy.mm.dd-hh.mm.ss), Results file name (same as the .gpr file name), StorageCD, and Extraction comments.

<u>Pre-processing with Excel Templates</u>

Following analysis of the image and extraction of the data, the data from each hybridization was pre-processed to extract data that was entered into the database and subsequently used for analysis. The complete GPR file produced by the feature extraction in GenePix was imported into an excel file pre-processing template or processed using a AWK script. Both programs used the same processing logic and produce identical results. The same excel template or AWK script was used to process each

GPR file. The template performs a series of calculations on the data to differentiate poor features from others and to combine duplicate or triplicate feature data into a single data point for each probe.

The data columns used in the pre-processing were: Oligo ID, F633 Median (median value from all the pixels in the feature for the Cy5 dye), B633 Median (the median value of all the pixels in the local background of the selected feature for Cy5), B633 SD (the standard deviation of the values for the pixels in the local background of the selected feature for Cy5), F532 Median (median value from all the pixels in the feature for the Cy3 dye), B532 Median (the median value of all the pixels in the local background of the selected feature for Cy3), B532 SD (the standard deviation of the values for the pixels in the local background of the selected feature for Cy3), and Flags. The GenePix Flags column contains the flags set during feature extraction. "-75" indicates there were no features printed on the array in that position, "-50" indicates that GenePix could not differentiate the feature signal from the local background, and "-100" indicates that the user marked the feature as bad.

Once imported, the data associated with features with -75 flags was not used. Then the median of B633 SD and B532 SD were calculated over all features with a flag value of "0". The minimum values of B633 Median and B532 Median were identified, considering only those values associated with a flag value of "0". For each feature, the signal to noise ratio (S/N) was calculated for both dyes by taking the fluorescence signal minus the local background (BGSS) and dividing it by the standard deviation of the local background:

$$S/N = \frac{F_i - B_i}{SDB_i}$$

If the S/N was less than 3, then an adjusted background-subtracted signal was calculated as the fluorescence minus the minimum local background on the slide. An adjusted S/N was then calculated as the adjusted background subtracted signal divided by the median noise over all features for that channel. If the adjusted S/N was greater than three and the original S/N were less than three, a flag of 25 was set for the Cy5 channel, a flag of 23 was set for the Cy3 channel, and if both met these criteria, then a flag of 28 was set. If both the adjusted S/N and the original S/N were less than three, then a flag of 65 was set for Cy5, 63 set for Cy3, and 68 set if both dye channels had an adjusted S/N less than three. All signal to noise calculations, adjusted background-subtracted signal, and adjusted S/N were calculated for each dye channel. If the BGSS value was greater than or equal to 64000, a flag was set to indicate saturation; 55 for Cy5, 53 for Cy3, 58 for both.

The BGSS used for further calculations was the original BGSS if the original S/N was greater than or equal to three. If the original S/N ratio was less than three and the adjusted S/N ratio was greater than or equal to three, then the adjusted BGSS was used. If the adjusted S/N ratio was less than three, then the adjusted BGSS was used, but with knowledge of the flag status.

To facilitate comparison among arrays, the Cy3 and Cy5 data were scaled. The log of the ratio of Green/Red was determined for all features. The median log ratio value for good features (Flags 0,

23, 25, 28, 63) was determined. The feature values were scaled using the following formula: Log_Scaled_Feature_Ratio = Log_Feature_Ratio – Median_Log_Ratio.

The flag setting for each feature was used to determine the expression ratio for each probe, a choice of one, two or three features. If all features had flag settings in the same category (categories=negatives, 0 to 28, 53-58, and 63-68), then the average of the three scaled, anti log feature ratios was calculated. If the three features did not have flags in the same category, then the feature or features with the best quality flags were used (0>25>23>28>55>53>58>65>63>68). Features with negative flags were never used. When the best flags were two or three features in the same category, the anti log average was used. If a single feature had a better flag category than the other two then the anti log of that feature ratio was used.

Once the probe expression ratios were calculated from the one, two, or three features, the log of the scaled, averaged ratios was taken as described below and stored for use in analyzing the data. Whichever features were used to calculate the probe value, the flag from those features was carried forward and stored as the flag value for that probe. 2 different data sets can be used for analysis. Flagged data uses all values, including those with flags. Filtered data sets are created by removing flagged data from the set before analysis.

Example 8: Real-time PCR validation of array expression results

Leukocyte microarray gene expression was used to discover expression markers and diagnostic gene sets for clinical outcomes. It is desirable to validate the gene expression results for each gene using a more sensitive and quantitative technology such as real-time PCR. Further, it is possible for the diagnostic nucleotide sets to be implemented as a diagnostic test as a real-time PCR panel. Alternatively, the quantitative information provided by real-time PCR validation can be used to design a diagnostic test using any alternative quantitative or semi-quantitative gene expression technology.

To validate the results of the microarray experiments we used real-time, or kinetic, PCR. In this type of experiment the amplification product is measured during the PCR reaction. This enables the researcher to observe the amplification before any reagent becomes rate limiting for amplification. In kinetic PCR the measurement is of C_T (threshold cycle) or C_P (crossing point). This measurement (C_T=C_P) is the point at which an amplification curve crosses a threshold fluorescence value. The threshold is set to a point within the area where all of the reactions were in their linear phase of amplification. When measuring C_T, a lower C_T value is indicative of a higher amount of starting material since an earlier cycle number means the threshold was crossed more quickly.

Several fluorescence methodologies are available to measure amplification product in real-time PCR. Taqman (Applied BioSystems, Foster City, CA) uses fluorescence resonance energy transfer (FRET) to inhibit signal from a probe until the probe is degraded by the sequence specific binding and Taq 3′ exonuclease activity. Molecular Beacons (Stratagene, La Jolla, CA) also use FRET technology, whereby the fluorescence is measured when a hairpin structure is relaxed by the specific probe binding to the amplified DNA. The third commonly used chemistry is Sybr Green, a DNA-

binding dye (Molecular Probes, Eugene, OR). The more amplified product that is produced, the higher the signal. The Sybr Green method is sensitive to non-specific amplification products, increasing the importance of primer design and selection. Other detection chemistries can also been used, such as ethedium bromide or other DNA-binding dyes and many modifications of the fluorescent dye/quencher dye Taqman chemistry.

Sample prep and cDNA synthesis

The inputs for real time PCR reaction are gene-specific primers, cDNA from specific patient samples, and standard reagents. The cDNA was produced from mononuclear RNA (prepared as in example 1) by reverse transcription using Oligo dT primers (Invitrogen, 18418-012) and random hexamers (Invitrogen, 48190-011) at a final concentration of 0.5ng/µl and 3ng/µl respectively. For the first strand reaction mix, 0.5 µg of mononuclear total RNA or 2 µg of whole blood RNA and 1 µl of the Oligo dT/ Random Hexamer Mix, were added to water to a final volume of 11.5 µl. The sample mix was then placed at 70°C for 10 minutes. Following the 70°C incubation, the samples were chilled on ice, spun down, and 88.5 µl of first strand buffer mix dispensed into the reaction tube. The final first strand buffer mix produced final concentrations of 1X first strand buffer (Invitrogen, Y00146, Carlsbad, CA), 10 mM DTT (Invitrogen, Y00147), 0.5 mM dATP (NEB, N0440S, Beverly, MA), 0.5 mM dGTP (NEB, N0442S), 0.5mM dTTP (NEB, N0443S), 0.5 mM dCTP (NEB, N0441S), 200U of reverse transcriptase (Superscript II, Invitrogen, 18064-014), and 18U of RNase inhibitor (RNAGaurd Amersham Pharmacia, 27-0815-01, Piscataway, NJ). The reaction was incubated at 42°C for 90 minutes. After incubation the enzyme was heat inactivated at 70°C for 15 minutes, 2 U of RNAse H added to the reaction tube, and incubated at 37°C for 20 minutes.

PRIMER DESIGN

Two methods were used to design primers. The first was to use the software, Primer Express[tm] and recommendations for primer design that are provided with the GeneAmp® 7700 Sequence Detection System supplied by Applied BioSystems (Foster City, CA). The second method used to design primers was the PRIMER3 ver 0.9 program that is available from the Whitehead Research Institute, Cambridge, Massachusetts at the web site genome.wi.mit.edu/genome_software/other/primer3.html. The program can also be accessed on the World Wide Web at the web site genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi. Primers and Taqman/hybridization probes were designed as described below using both programs.

The Primer Express literature explains that primers should be designed with a melting temperature between 58 and 60 degrees C. while the Taqman probes should have a melting temperature of 68 to 70 under the salt conditions of the supplied reagents. The salt concentration is fixed in the software. Primers should be between 15 and 30 basepairs long. The primers should produce and amplicon in size between 50 and 150 base pairs, have a C-G content between 20% and 80%, have no more than 4 identical base pairs next to one another, and no more than 2 C's and G's in the last 5 bases of the 3' end. The probe cannot have a G on the 5' end and the strand with the fewest G's should be used for the probe.

Primer3 has a large number of parameters. The defaults were used for all except for melting temperature and the optimal size of the amplicon was set at 100 bases. One of the most critical is salt

concentration as it affects the melting temperature of the probes and primers. In order to produce primers and probes with melting temperatures equivalent to Primer Express, a number of primers and probes designed by Primer Express were examined using PRIMER3. Using a salt concentration of 50 mM these primers had an average melting temperature of 3.7 degrees higher than predicted by Primer Express. In order to design primers and probes with equivalent melting temperatures as Primer Express using PRIMER3, a melting temperature of 62.7 plus/minus 1.0 degree was used in PRIMER3 for primers and 72.7 plus/minus 1.0 degrees for probes with a salt concentration of 50 mM.

The C source code for Primer3 was downloaded and complied on a Sun Enterprise 250 server using the GCC complier. The program was then used from the command line using a input file that contained the sequence for which we wanted to design primers and probes along with the input parameters as described by help files that accompany the software. Using scripting it was possible to input a number of sequences and automatically generate a number of possible probes and primers.

Primers for β-Actin (Beta Actin, Genbank Locus: NM_001101)and β-GUS: glucuronidase, beta, (GUSB, Genbank Locus: NM_000181), two reference genes, were designed using both methods and are shown here as examples:

The first step was to mask out repetitive sequences found in the mRNA sequences using RepeatMasker program that can be accessed at: the web site repeatmasker.genome.washington.edu/cgi-bin/RepeatMasker (Smit, AFA & Green, P "RepeatMasker" at the web site ftp.genome.washington.edu/RM/RepeatMasker.html).

The last 500 basepairs on the last 3' end of masked sequence was then submitted to PRIMER3 using the following exemplary input sequences:

```
PRIMER_SEQUENCE_ID=>ACTB Beta Actin (SEQ ID NO:1049)
SEQUENCE=TTGGCTTGACTCAGGATTTAAAAACTGGAACGGTGAAGGTGACAGCAGTCGGTTGGACGA
GCATCCCCCAAAGTTCACAATGTGGCCGAGGACTTTGATTGCACATTGTTGTTTTTTAATAGTCATTCC
AAATATGAGATGCATTGTTACAGGAAGTCCCTTGCCATCCTAAAAGCACCCCACTTCTCTCTAAGGAGA
ATGGCCCAGTCCTCTCCCAAGTCCACACAGGGGAGGGATAGCATTGCTTTCGTGTAAATTATGTAATGC
AAAATTTTTTTAATCTTCGCCTTAATCTTTTTTATTTTGTTTTATTTTGAATGATGAGCCTTCGTGCCC
CCCCTTCCCCCTTTTTTCCCCCAACTTGAGATGTATGAAGGCTTTTGGTCTCCCTGGGAGTGGGTGGAG
GCAGCCGGGCTTACCTGTACACTGACTTGAGACCAGTTGAATAAAAGTGCACACCTTA
```

```
PRIMER_SEQUENCE_ID=>GUSB (SEQ ID NO:1050)
SEQUENCE=GAAGAGTACCAGAAAAGTCTGCTAGAGCAGTACCATCTGGGTCTGGATCAAAAACGCAGA
AAATATGTGGTTGGAGAGCTCATTTGGAATTTTGCCGATTTCATGACTGAACAGTCACCGACGAGAGTG
CTGGGGAATAAAAAGGGGATCTTCACTCGGCAGAGACAACCAAAAAGTGCAGCGTTCCTTTTGCGAGAG
AGATACTGGAAGATTGCCAATGAAACCAGGTATCCCCACTCAGTAGCCAAGTCACAATGTTTGGAAAAC
AGCCCGTTTACTTGAGCAAGACTGATACCACCTGCGTGTCCCTTCCTCCCCGAGTCAGGGCGACTTCCA
CAGCAGCAGAACAAGTGCCTCCTGGACTGTTCACGGCAGACCAGAACGTTTCTGGCCTGGGTTTTGTGG
TCATCTATTCTAGCAGGGAACACTAAAGGTGGAAATAAAAGATTTTCTATTATGGAAATAAAGAGTTGG
CATGAAAGTCGCTACTG
```

After running PRIMER3, 100 sets of primers and probes were generated for ACTB and GUSB. From this set, nested primers were chosen based on whether both left primers could be paired with both right primers and a single Taqman probe could be used on an insert of the correct size. With more experience we have decided not use the mix and match approach to primer selection and just use several of the top pairs of predicted primers.

For ACTB this turned out to be:
Forward 75 CACAATGTGGCCGAGGACTT(SEQ ID NO:1051),
Forward 80 TGTGGCCGAGGACTTTGATT(SEQ ID NO:1052),
Reverse 178 TGGCTTTTAGGATGGCAAGG(SEQ ID NO:1053), and
Reverse 168 GGGGGCTTAGTTTGCTTCCT(SEQ ID NO:1054).

Upon testing, the F75 and R178 pair worked best.

For GUSB the following primers were chosen:
Forward 59 AAGTGCAGCGTTCCTTTTGC(SEQ ID NO:1055),
Forward 65 AGCGTTCCTTTTGCGAGAGA (SEQ ID NO:1056),
Reverse 158 CGGGCTGTTTTCCAAACATT (SEQ ID NO:1057), and
Reverse 197 GAAGGGACACGCAGGTGGTA (SEQ ID NO:1058).

No combination of these GUSB pairs worked well.

In addition to the primer pairs above, Primer Express predicted the following primers for GUSB: Forward 178 TACCACCTGCGTGTCCCTTC (SEQ ID NO:1059) and Reverse 242 GAGGCACTTGTTCTGCTGCTG (SEQ ID NO:1060). This pair of primers worked to amplify the GUSB mRNA.

The parameters used to predict these primers in Primer Express were:
Primer Tm: min 58, Max=60, opt 59, max difference=2 degrees
Primer GC: min=20% Max =80% no 3' G/C clamp
Primer: Length: min=9 max=40 opt=20
Amplicon: min Tm=0 max Tm=85
min = 50 bp max = 150 bp
Probe: Tm 10 degrees > primers, do not begin with a G on 5' end
Other: max base pair repeat = 3
max number of ambiguous residues = 0
secondary structure: max consecutive bp = 4, max total bp = 8
Uniqueness: max consecutive match = 9
max % match = 75
max 3' consecutive match = 7

Granzyme B is a marker of transplant rejection.

For Granzyme B the following sequence (NM_004131) (SEQ ID:1061) was used as input for Primer3 :

GGGGACTCTGGAGGCCCTCTTGTGTGTAACAAGGTGGCCCAGGGCATTGT
CTCCTATGGACGAAACAATGGCATGCCTCCACGAGCCTGCACCAAAGTCT
CAAGCTTTGTACACTGGATAAAGAAAACCATGAAACGCTACTAACTACAG
GAAGCAAACTAAGCCCCCGCTGTAATGAAACACCTTCTCTGGAGCCAAGT
CCAGATTTACACTGGGAGAGGTGCCAGCAACTGAATAAATACCTCTCCCA
GTGTAAATCTGGAGCCAAGTCCAGATTTACACTGGGAGAGGTGCCAGCAA
CTGAATAAATACCTCTTAGCTGAGTGG

For Granzyme B the following primers were chosen for testing:
Forward 81 ACGAGCCTGCACCAAAGTCT (SEQ ID NO:1062)
Forward 63 AAACAATGGCATGCCTCCAC (SEQ ID NO:1063)
Reverse 178 TCATTACAGCGGGGGCTTAG (SEQ ID NO:1064)
Reverse 168 GGGGGCTTAGTTTGCTTCCT (SEQ ID NO:1065)

Testing demonstrated that F81 and R178 worked well.

Using this approach, primers were designed for all the genes that were shown to have expression patterns that correlated with allograft rejection. Primers can be designed from any region of a target gene using this approach.

PRIMER ENDPOINT TESTING

Primers were first tested to examine whether they would produce the correct size product without non-specific amplification. The standard real-time PCR protocol was used without the Rox and Sybr green dyes. Each primer pair was tested on cDNA made from universal mononuclear leukocyte reference RNA that was produced from 50 individuals as described in Example 2 (R50).

The PCR reaction consisted of 1X RealTime PCR Buffer (Ambion, Austin, TX), 2mM MgCl2 (Applied BioSystems, B02953), 0.2mM dATP (NEB), 0.2mM dTTP (NEB), 0.2mM dCTP (NEB), 0.2mM dGTP (NEB), .625U AmpliTaq Gold (Applied BioSystems, Foster City, CA), 0.3μM of each primer to be used (Sigma Genosys, The Woodlands, TX), 5μl of the R50 reverse-transcription reaction and water to a final volume of 19μl.

Following 40 cycles of PCR, 10 microliters of each product was combined with Sybr green at a final dilution of 1:72,000. Melt curves for each PCR product were determined on an ABI 7900 (Applied BioSystems, Foster City, CA), and primer pairs yielding a product with one clean peak were chosen for further analysis. One microliter of the product from these primer pairs was examined by agarose gel electrophoresis on an Agilent Bioanalyzer, DNA1000 chip (Palo Alto, CA). Results for 2 genes are shown in Figure 6. From the primer design and the sequence of the target gene, one can calculate the expected size of the amplified DNA product. Only primer pairs with amplification of the desired product and minimal amplification of contaminants were used for real-time PCR. Primers that produced multiple products of different sizes are likely not specific for the gene of interest and may amplify multiple genes or chromosomal loci.

PRIMER OPTIMIZATION/EFFICIENCY

Once primers passed the end-point PCR, the primers were tested to determine the efficiency of the reaction in a real-time PCR reaction. cDNA was synthesized from starting total RNA as described above. A set of 5 serial dilutions of the R50 reverse-transcribed cDNA (as described above) were made in water: 1:10, 1:20, 1:40, 1:80, and 1:160.

The Sybr Green real-time PCR reaction was performed using the Taqman PCR Reagent kit (Applied BioSystems, Foster City, CA, N808-0228). A master mix was made that consisted of all reagents except the primes and template. The final concentration of all ingredients in the reaction was 1X Taqman Buffer A (Applied BioSystems), 2mM MgCl2 (Applied BioSystems), 200μM dATP (Applied BioSystems), 200μM dCTP (Applied BioSystems), 200μM dGTP (Applied BioSystems), 400μM dUTP (Applied BioSystems), 1:400,000 diluted Sybr Green dye (Molecular Probes), 1.25U AmpliTaq Gold (Applied BioSystems). The PCR master mix was dispensed into two, light-tight tubes. Each β-Actin primer F75 and R178 (Sigma-Genosys, The Woodlands, TX), was added to one tube of PCR master mix and Each β-GUS primer F178 and R242 (Sigma-Genosys), was added to the other tube of PCR master mix to a final primer concentration of 300nM. 45μl of the β-Actin or β-GUS master mix was dispensed into wells, in a 96-well plate (Applied BioSystems). 5μl of the template

dilution series was dispensed into triplicate wells for each primer. The reaction was run on an ABI 7900 Sequence Detection System (Applied BioSystems) with the following conditions: 10 min. at 95°C; 40 cycles of 95°C for 15 sec, 60°C for 1 min; followed by a disassociation curve starting at 50°C and ending at 95°C. .

The Sequence Detection System v2.0 software was used to analyze the fluorescent signal from each well. The high end of the baseline was adjusted to between 8 and 20 cycles to reduce the impact on any data curves, yet be as high as possible to reduce baseline drift. A threshold value was selected that allowed the majority of the amplification curves to cross the threshold during the linear phase of amplification. The disassociation curve for each well was compared to other wells for that marker. This comparison allowed identification of "bad" wells, those that did not amplify, that amplified the wrong size product, or that amplified multiple products. The cycle number at which each amplification curve crossed the threshold (C_T) was recorded and the file transferred to MS Excel for further analysis. The C_T values for triplicate wells were averaged. The data were plotted as a function of the $\log_{10}$ of the calculated starting concentration of RNA. The starting RNA concentration for each cDNA dilution was determined based on the original amount of RNA used in the RT reaction, the dilution of the RT reaction, and the amount used (5 µl) in the real-time PCR reaction. For each gene, a linear regression line was plotted through all of the dilutions series points. The slope of the line was used to calculate the efficiency of the reaction for each primer set using the equation:

$$E = 10^{\left(-1/_{\text{slope}}\right)} - 1$$

Using this equation (Pfaffl 2001, Applied Biosystems User Bulletin #2), the efficiency for these β-actin primers is 1.28 and the efficiency for these β-GUS primers is 1.14 (Figure 6). This efficiency was used when comparing the expression levels among multiple genes and multiple samples. This same method was used to calculate reaction efficiency for primer pairs for each gene studied. A primer pair was considered successful if the efficiency was reproducibly determined to be between 0.7 and 2.4.

SYBR-GREEN ASSAYS

Once markers passed the Primer Efficiency QPCR (as stated above), they were used in real-time PCR assays. Patient RNA samples were reverse-transcribed to cDNA (as described above) and 1:10 dilutions made in water. In addition to the patient samples, a no template control (NTC) and a pooled reference RNA (see example 2) described in were included on every plate.

The Sybr Green real-time PCR reaction was performed using the Taqman Core PCR Reagent kit (Applied BioSystems, Foster City, CA, N808-0228). A master mix was made that consisted of all reagents except the primers and template. The final concentration of all ingredients in the reaction was 1X Taqman Buffer A (Applied BioSystems), 2mM MgCl2 (Applied BioSystems), 200µM dATP (Applied BioSystems), 200µM dCTP (Applied BioSystems), 200µM dGTP (Applied BioSystems), 400µM dUTP (Applied BioSystems), 1:400,000 diluted Sybr Green dye (Molecular Probes), 1.25U AmpliTaq Gold (Applied BioSystems). The PCR master mix was aliquotted into eight light-tight

tubes, one for each marker to be examined across a set of samples. The optimized primer pair for each marker was then added to the PCR master mix to a final primer concentration of 300nM. 18µl of the each marker master mix was dispensed into wells in a 384well plate (Applied BioSystems). 2µl of the 1:10 diluted control or patient cDNA sample was dispensed into triplicate wells for each primer pair. The reaction was run on an ABI 7900 Sequence Detection System (Applied BioSystems) using the cycling conditions described above.

The Sequence Detection System v2.0 software (Applied BioSystems) was used to analyze the fluorescent signal from each well. The high end of the baseline was adjusted to between 8 and 20 cycles to reduce the impact on any data curves, yet be as high as possible to reduce baseline drift. A threshold value was selected that allowed the majority of the amplification curves to cross the threshold during the linear phase of amplification. The disassociation curve for each well was compared to other wells for that marker. This comparison allowed identification of "bad" wells, those that did not amplify, that amplified the wrong size product, or that amplified multiple products. The cycle number at which each amplification curve crossed the threshold (C_T) was recorded and the file transferred to MS Excel for further analysis. The C_T value representing any well identified as bad by analysis of disassociation curves was deleted. The C_T values for triplicate wells were averaged. A standard deviation (Stdev) and a coefficient of variation (CV) were calculated for the triplicate wells. If the CV was greater than 2, an outlier among the three wells was identified and deleted. Then the average was re-calculated. In each plate, ΔC_T was calculated for each marker-control combination by subtracting the average C_T of the target marker from the average C_T of the control (β-Actin or β-GUS). The expression relative to the control marker was calculated by taking two to the power of the ΔC_T of the target marker. For example, expression relative to β-Actin was calculated by the equation:

$$ErA = 2^{\left(C_{T,Actin} - C_{T,target}\right)}$$

All plates were run in duplicate and analyzed in the same manner. The percent variation was determined for each sample-marker combination (relative expression) by taking the absolute value of the value of the RE for the second plate from the RE for the first plate, and dividing that by the average. If more than 25% of the variation calculations on a plate are greater than 50%, then a third plate was run.

TAQMAN PROTOCOL

Real-time PCR assays were also done using Taqman PCR chemistry.

The Taqman real-time PCR reaction was performed using the Taqman Universal PCR Master Mix (Applied BioSystems, Foster City, CA, #4324018). The master mix was aliquoted into eight, light-tight tubes, one for each marker. The optimized primer pair for each marker was then added to the correctly labeled tube of PCR master mix. A FAM/TAMRA dual-labeled Taqman probe (Biosearch Technologies, Navoto, CA, DLO-FT-2) was then added to the correctly labeled tube of PCR master mix. Alternatively, different combinations of fluorescent reporter dyes and quenchers can be used such that the absorption wavelength for the quencher matches the emission wavelength for the reporter, as

shown in table 4. 18µl of the each marker master mix was dispensed into a 384well plate (Applied BioSystems). 2µl of the template sample was dispensed into triplicate wells for each primer pair. The final concentration of each reagent was: 1X TaqMan Universal PCR Master Mix, 300nM each primer, 0.25nM probe, 2µl 1:10 diluted template. The reaction was run on an ABI 7900 Sequence Detection System (Applied Biosystems) using standard conditions (95°C for 10 min., 40 cycles of 95°C for 15 sec, 60°C for 1 min.).

The Sequence Detector v2.0 software (Applied BioSystems) was used to analyze the fluorescent signal from each well. The high end of the baseline was adjusted to between 8 and 20 cycles to reduce the impact on any data curves, yet be as high as possible to reduce baseline drift. A threshold value was selected that allowed most of the amplification curves to cross the threshold during the linear phase of amplification. The cycle number at which each amplification curve crossed the threshold (C_T) was recorded and the file transferred to MS Excel for further analysis. The C_T values for triplicate wells were averaged. The C_T values for triplicate wells were averaged. A standard deviation (Stdev) and a coefficient of variation (CV) were calculated for the triplicate wells. If the CV was greater than 2, an outlier among the three wells was identified and deleted. Then the average was re-calculated. In each plate, ΔC_T was calculated for each marker-control combination by subtracting the average C_T of the target marker from the average C_T of the control (*β*-Actin or *β*-GUS). The expression relative to the control marker was calculated by taking two to the power of the ΔC_T of the target marker. All plates were run in duplicate and analyzed in the same manner. The percent variation was determined for each sample-marker combination (relative expression) by taking the absolute value of the value of the RE for the second plate from the RE for the first plate, and dividing that by the average. If more than 25% of the variation calculations on a plate are greater than 50%, then a third plate was run.

BI-PLEXING

Variation of real-time PCR assays can arise from unequal amounts of RNA starting material between reactions. In some assays, to reduce variation, the control gene amplification was included in the same reaction well as the target gene. To differentiate the signal from the two genes, different fluorescent dyes were used for the control gene. *β*-Actin was used as the control gene and the TaqMan probe used was labeled with the fluorescent dye VIC and the quencher TAMRA (Biosearch Technologies, Navoto, CA, DLO-FT-2). Alternatively, other combinations of fluorescent reporter dyes and quenchers (table 4) can be used as long as the emission wavelength of the reporter for the control gene is sufficiently different from the wavelength of the reporter dye used for the target. The control gene primers and probe were used at limiting concentrations in the reaction (150 nM primers and 0.125 nM probe) to ensure that there were enough reagents to amplify the target marker. The plates were run under the same protocol and the data are analyzed in the same way, but with a separate baseline and threshold for the VIC signal. Outliers were removed as above from both the FAM and VIC signal channels. The expression relative to control was calculated as above, using the VIC signal from the control gene.

$$ErA = 2^{(C_{T,VIC} - C_{T,FAM})}$$

ABSOLUTE QUANTITATION

Instead of calculating the expression relative to a reference marker, an absolute quantitation can be performed using real-time PCR. To determine the absolute quantity of each marker, a standard curve is constructed using serial dilutions from a known amount of template for each marker on the plate. The standard curve may be made using cloned genes purified from bacteria or using synthetic complimentary oligonucleotides. In either case, a dilution series that covers the expected range of expression is used as template in a series of wells in the plate. From the average C_T values for these known amounts of template a standard curve can be plotted. From this curve the C_T values for the unknowns are used to identify the starting concentration of cDNA. These absolute quantities can be compared between disease classes (i.e. rejection vs. no-rejection) or can be taken as expression relative to a control gene to correct for variation among samples in sample collection, RNA purification and quantification, cDNA synthesis, and the PCR amplification.

CELL TYPE SPECIFIC EXPRESSION

Some markers are expressed only in specific types of cells. These markers may be useful markers for differentiation of rejection samples from no-rejection samples or may be used to identify differential expression of other markers in a single cell type. A specific marker for cytotoxic T-lymphocytes (such as CD8) can be used to identify differences in cell proportions in the sample. Other markers that are known to be expressed in this cell type can be compared to the level of CD8 to indicate differential gene expression within CD8 T-cells.

Control genes for PCR

As discussed above, PCR expression measurements can be made as either absolute quantification of gene expression using a standard curve or relative expression of a gene of interest compared to a control gene. In the latter case, the gene of interest and the control gene are measured in the same sample. This can be done in separate reactions or in the same reaction (biplex format, see above). In either case, the final measurement for expression of a gene is expressed as a ratio of gene expression to control gene expression. It is important for a control gene to be constitutively expressed in the target tissue of interest and have minimal variation in expression on a per cell basis between individuals or between samples derived from an individual. If the gene has this type of expression behavior, the relative expression ratio will help correct for variability in the amount of sample RNA used in an assay. In addition, an ideal control gene has a high level of expression in the sample of interest compared to the genes being assayed. This is important if the gene of interest and control gene are used in a biplex format. The assay is set up so that the control gene reaches its threshold Ct value early and its amplification is limited by primers so that it does not compete for limiting reagents with the gene of interest.

To identify an ideal control gene for an assay, a number of genes were tested for variability between samples and expression in both mononuclear RNA samples and whole blood RNA samples using the RNA procurement and preparation methods and real-time PCR assays described above. 6 whole-blood and 6 mononuclear RNA samples from transplant recipients were tested. The intensity levels and variability of each gene in duplicate experiments on both sample types are shown in figure 8.

Based on criteria of low variability and high expression across samples, β-actin, 18s, GAPDH, b2microglobulin were found to be good examples of control genes for the PAX samples. A single control gene may be incorporated as an internal biplex control is assays.

Controlling for variation in real time PCR

Due to differences in reagents, experimenters, and preparation methods, and the variability of pipetting steps, there is significant plate-to-plate variation in real-time PCR experiments. This variation can be reduced by automation (to reduce variability and error), reagent lot quality control, and optimal data handling. However, the results on replicate plates are still likely to be different since they are run in the machine at different times.

Variation can also enter in data extraction and analysis. Real-time PCR results are measured as the time (measured in PCR cycles) at which the fluorescence intensity (ΔRn in Applied Biosystems SDS v2.1 software) crosses a user-determined threshold (CT). When performing relative quantification, the CT value for the target gene is subtracted from the CT value for a control gene. This difference, called ΔCT, is the value compared among experiments to determine whether there is a difference between samples. Variation in setting the threshold can introduce additional error. This is especially true in the duplexed experimental format, where both the target gene and the control gene are measured in the same reaction tube. Duplexing is performed using dyes specific to each of the two genes. Since two different fluorescent dyes are used on the plate, two different thresholds are set. Both of these thresholds contribute to each ΔCT. Slight differences in the each dye's threshold settings (relative to the other dye) from one plate to the next can have significant effects on the ΔCT.

There are several methods for setting the threshold for a PCR plate. Older versions of SDS software (Applied Biosystems) determine the average baseline fluorescence for the plate and the standard deviation of the baseline. The threshold is set to 10x the standard deviation of the baseline. In SDS 2.0 the users must set the baseline by themselves. Software from other machine manufacturers either requires the user to set the threshold themselves or uses different algorithms. The latest version of the SDS software (SDS 2.1) contains Automatic baseline and threshold setting. The software sets the baseline separately for each well on the plate using the ΔRn at cycles preceding detectable levels.

Variability among plates is dependent on reproducible threshold setting. This requires a mathematical or experimental data driven threshold setting protocol. Reproducibly setting the threshold according to a standard formula will minimize variation that might be introduced in the threshold setting process.

Additionally, there may be experimental variation among plates that can be reduced by setting the threshold to a component of the data. We have developed a system that uses a set of reactions on each plate that are called the threshold calibrator (TCb). The TCb wells are used to set the threshold on all plates.

1. The TCb wells contain a template, primers, and probes that are common among all plates within an experiment.

2. The threshold is set within the minimum threshold and maximum threshold determined above.

3. The threshold is set to a value in this range that results in the average CT value for the TCb wells to be the same on all plates.

Example 9: Correlation and Classification Analysis

After generation and processing of expression data sets from microarrays as described in Example 7, a log ratio value is used for most subsequent analysis. This is the logarithm of the expression ratio for each gene between sample and universal reference. The processing algorithm assigns a number of flags to data that are of low signal to noise, saturated signal or are in some other way of low or uncertain quality. Correlation analysis can proceed with all the data (including the flagged data) or can be done on filtered data sets where the flagged data is removed from the set. Filtered data should have less variability and noise and may result in more significant or predictive results. Flagged data contains all information available and may allow discovery of genes that are missed with the filtered data set.

After filtering the data for quality as described above and in example 7, missing data are common in microarray data sets. Some algorithms don't require complete data sets and can thus tolerate missing values. Other algorithms are optimal with or require imputed values for missing data. Analysis of data sets with missing values can proceed by filtering all genes from the analysis that have more than 5%, 10%, 20%, 40%, 50%, 60% or other % of values missing across all samples in the analysis. Imputation of data for missing values can be done by a variety of methods such as using the row mean, the column mean, the nearest neighbor or some other calculated number. Except when noted, default settings for filtering and imputation were used to prepare the data for all analytical software packages.

In addition to expression data, clinical data are included in the analysis. Continuous variables, such as the ejection fraction of the heart measured by echocardiography or the white blood cell count can be used for correlation analysis. Any piece of clinical data collected on study subjects can be used in a correlation or classification analysis. In some cases, it may be desirable to take the logarithm of the values before analysis. These variables can be included in an analysis along with gene expression values, in which case they are treated as another "gene". Sets of markers can be discovered that work to diagnose a patient condition and these can include both genes and clinical parameters. Categorical variables such as male or female can also be used as variables for correlation analysis. For example, the sex of a patient may be an important splitter for a classification tree.

Clinical data are used as supervising vectors (dependent variables) for the significance or classification analysis of expression data. In this case, clinical data associated with the samples are used to divide samples in to clinically meaningful diagnostic categories for correlation or classification analysis. For example, pathologic specimens from kidney biopsies can be used to divide lupus patients into groups with and without kidney disease. A third or more categories can also be included (for example "unknown" or "not reported"). After generation of expression data and definition of supervising vectors, correlation, significance and classification analysis are used to determine which set of genes and set of genes are most appropriate for diagnosis and classification of patients and patient samples.

Two main types of expression data analyses are commonly performed on the expression data with differing results and purposes. The first is significance analyses or analyses of difference. In this case, the goal of the analysis is to identify genes that are differentially expressed between sample groups and to assign a statistical confidence to those genes that are identified. These genes may be markers of the disease process in question and are further studied and developed as diagnostic tools for the indication.

The second major type of analysis is classification analysis. While significance analysis identifies individual genes that are differentially expressed between sample groups, classification analysis identifies gene sets and an algorithm for their gene expression values that best distinguish sample (patient) groups. The resulting gene expression panel and algorithm can be used to create and implement a diagnostic test. The set of genes and the algorithm for their use as a diagnostic tool are often referred to herein as a "model". Individual markers can also be used to create a gene expression diagnostic model. However, multiple genes (or gene sets) are often more useful and accurate diagnostic tools.

Significance analysis for microarrays (SAM)

Significance analysis for microarrays (SAM) (Tusher 2001) is a method through which genes with a correlation between their expression values and the response vector are statistically discovered and assigned a statistical significance. The ratio of false significant to significant genes is the False Discovery Rate (FDR). This means that for each threshold there are some number of genes that are called significant, and the FDR gives a confidence level for this claim. If a gene is called differentially expressed between two classes by SAM, with a FDR of 5%, there is a 95% chance that the gene is actually differentially expressed between the classes. SAM will identify genes that are differentially expressed between the classes. The algorithm selects genes with low variance within a class and large variance between classes. The algorithm may not identify genes that are useful in classification, but are not differentially expressed in many of the samples. For example, a gene that is a useful marker for disease in women and not men, may not be a highly significant marker in a SAM analysis, but may be useful as part of a gene set for diagnosis of a multi-gene algorithm.

After generation of data from patient samples and definition of categories using clinical data as supervising vectors, SAM is used to detect genes that are likely to be differentially expressed between the groupings. Those genes with the highest significance can be validated by real-time PCR (Example 8) or can be used to build a classification algorithm as described here.

Classification

Classification algorithms are used to identify sets of genes and formulas for the expression levels of those genes that can be applied as diagnostic and disease monitoring tests. The same classification algorithms can be applied to all types of expression and proteomic data, including microarray and PCR based expression data. The discussion below describes the algorithms that were used and how they were used.

Classification and Regression Trees (CART) is a decision tree classification algorithm (Breiman 1984). From gene expression and or other data, CART can develop a decision tree for the classification of samples. Each node on the decision tree involves a query about the expression level of

one or more genes or variables. Samples that are above the threshold go down one branch of the decision tree and samples that are not go down the other branch. Genes from expression data sets can be selected for classification building with CART by significant differential expression in SAM analysis (or other significance test), identification by supervised tree-harvesting analysis, high fold change between sample groups, or known relevance to classification of the target diseases. In addition, clinical data can be used as independent variables for CART that are of known importance to the clinical question or are found to be significant predictors by multivariate analysis or some other technique. CART identifies predictive variables and their associated decision rules for classification (diagnosis). CART also identifies surrogates for each splitter (genes that are the next best substitute for a useful gene in classification). Analysis is performed in CART by weighting misclassification costs to optimize desired performance of the assay. For example, it may be most important that the sensitivity of a test for a given diagnosis be > 90%. CART models can be built and tested using 10 fold cross-validation or v-fold cross validation (see below). CART works best with a smaller number of variables (5-50).

Multiple Additive Regression Trees (Friedman, JH 1999, MART) is similar to CART in that it is a classification algorithm that builds decision trees to distinguish groups. MART builds numerous trees for any classification problem and the resulting model involves a combination of the multiple trees. MART can select variables as it build models and thus can be used on large data sets, such as those derived from an 8000 gene microarray. Because MART uses a combination of many trees and does not take too much information from any one tree, it resists over training. MART identifies a set of genes and an algorithm for their use as a classifier.

A Nearest Shrunken Centroids Classifier can be applied to microarray or other data sets by the methods described by Tibshirani et al. 2002. This algorithms also identified gene sets for classification and determines their 10 fold cross validation error rates for each class of samples. The algorithm determines the error rates for models of any size, from one gene to all genes in the set. The error rates for either or both sample classes can are minimized when a particular number of genes are used. When this gene number is determined, the algorithm associated with the selected genes can be identified and employed as a classifier on prospective sample.

For each classification algorithm and for significance analysis, gene sets and diagnostic algorithms that are built are tested by cross validation and prospective validation. Validation of the algorithm by these means yields an estimate of the predictive value of the algorithm on the target population. There are many approaches, including a 10 fold cross validation analysis in which 10% of the training samples are left out of the analysis and the classification algorithm is built with the remaining 90%. The 10% are then used as a test set for the algorithm. The process is repeated 10 times with 10% of the samples being left out as a test set each time. Through this analysis, one can derive a cross validation error which helps estimate the robustness of the algorithm for use on prospective (test) samples. Any % of the samples can be left out for cross validation (v-fold cross validation, LOOCV). When a gene set is established for a diagnosis with an acceptable cross validation error, this set of genes is tested using samples that were not included in the initial analysis (test samples). These samples may be taken from archives generated during the clinical study.

Alternatively, a new prospective clinical study can be initiated, where samples are obtained and the gene set is used to predict patient diagnoses.

Example 10: Assay sample preparation

In order to show that the leukocyte-specific markers of the present invention can be detected in whole blood, we collected whole blood RNA using the PAXgene whole blood collection, stabilization, and RNA isolation kit (PreAnalytix). Varying amounts of the whole blood RNA were used in the initial RT reaction (1, 2, 4, and 8ug), and varying dilutions of the different RT reactions were tested (1:5, 1:10, 1:20, 1:40, 1:80, 1:160). We did real-time PCR assays with primers specific to XDx's markers and showed that we can reliably detect these markers in whole blood.

Total RNA was prepared from 14 mononuclear samples (CPT, BD) paired with 14 whole blood samples (PAXgene, PreAnalytix) from transplant recipients. cDNA was prepared from each sample using 2μg total RNA as starting material. Resulting cDNA was diluted 1:10 and Sybr green real-time PCR assays were performed.

For real-time PCR assays, Ct values of 15-30 are desired for each gene. If a gene's Ct value is much above 30, the result may be variable and non-linear. For PAX sample, target RNA will be more dilute than in CPT samples. cDNA dilutions must be appropriate to bring Ct values to less than 30.

Ct values for the first 5 genes tested in this way are shown in the table below for both whole blood RNA (PAX) and mononuclear RNA (CPT).

Gene	Ct PAX	Ct CPT
CD20	27.41512	26.70474
4761	28.45656	26.52635
3096	29.09821	27.83281
GranzymeB	31.18779	30.56954
IL4	33.11774	34.8002
Actin	19.17622	18.32966
B-GUS	26.89142	26.92735

With one exception, the genes have higher Ct values in whole blood. Using this protocol, all genes can be detected with Cts <35. For genes found to have Ct values above 30 in target samples, less diluted cDNA may be needed.

Example 11: Identification and validation of gene expression markers for diagnosis and monitoring of lupus and autoimmune diseases.

Patients were enrolled in a clinical study as described in example 4.

58 peripheral blood samples from 22 patients meeting ACR criteria for SLE, 20 patients with rheumatoid arthritis (RA), 6 patients with osteoarthritis (OA), and 10 healthy donors (HD) were collected (see Table 1). Within 1 hour of collection, samples were processed by density gradient centrifugation and mononuclear cells were lysed and frozen using the technique described in example 1. Total RNA was prepared from cell pellets, amplified and labeled with fluorescent Cy3, and hybridized to a custom oligonucleotide microarray of 8143 DNA sequences selected by virtue of

differential or specific expression in activated or resting leukocytes (methods used are described in examples 5-7). After normalization, the log ratio of Cy3 (donor sample) to Cy5 (pooled reference leukocyte RNA) was used for analysis of gene sequences differentially expressed by the SLE samples versus all non-SLE samples. SLE samples were divided into classic or highly probable SLE diagnosis according to American College of Rheumatology clinical criteria or patients with the clinical diagnosis of SLE, but less with fewer classic signs or symptoms or recent treatment with increased immunosuppression.

Significance analysis of microarrays (SAM, Tusher et al. 2001) was used to identify genes that are differentially expressed between Lupus patients and controls (Example 9). These data are shown in Table 2A where genes are listed that were over- or under-expresed in SLE and control samples at various levels of false detection rates (FDR). Each of these genes may have a correlation to disease or disease activity. Also using the methods of example 9, Multiple Additive Regression Trees (MART) was also used to identify genes that best distinguished SLE from control samples in using multi-gene classification models (Table 2C). This analysis identifies gene sets and formulae that relate the genes to create a diagnostic or monitoring assay for lupus. Genes that are a part of a multi-gene algorithm with a low classification error rate on patient samples and a high level of importance in the algorithm are high priority gene markers.

Real-time PCR by techniques described in example 8 was used to confirm and quantify differential expression of selected gene sequences (Table 2B). PCR primers for all marker genes can be designed by these same methods. Many of the genes tested were validated and showed highly significant correlation or anti-correlation to lupus samples.

Hierarchical cluster analysis (Eisen et al. 1998, Example 9) identified both known and undescribed genes that are coexpressed with genes that showed promise in either the array or PCR data as being markers of SLE (Table 2D). Genes were thus grouped into pathways.

Among the genes identified by the significance and classification analysis, at many are members of the IFN regulatory factor or interferon-induced gene families. The interferon alpha family of molecules and pathways have been implicated in the pathogenesis of Lupus for some time. Patients treated with interferon alpha for chronic viral infections can have the side effect of autoimmune phenomena and Lupus. IFN-a levels are elevated in sera from patients with SLE, RA, Sjogren's syndrome, and scleroderma. IFN-a may also be involved in the very earliest events that initiate autoimmunity. Identification of the specific IFN-a gene products expressed in SLE may allow selective therapeutic targeting of pathogenic cytokines while sparing those IFN's that are protective in the setting of virus infection.

Table 1: Samples used in array and PCR expression profiling experiments

Patient	PrimaryDx	Dx1	Dx2	PCR
1	C	0	0	
2	C	0	0	x
3	C	0	2	x
4	C	0	0	
5	C	0	0	x
6	C	0	0	
7	C	0	0	x
8	C	0	0	x
9	C	0	0	
10	C	0	0	
11	OA	0	0	x
12	OA	0	0	x
13	OA	0	0	x
14	OA	0	0	x
15	OA	0	0	x
16	OA	0	0	
17	RA	0	0	x
18	RA	0	0	
19	RA	0	0	
20	RA	0	0	
21	RA	0	0	
22	RA	0	0	x
23	RA	0	0	
24	RA	0	0	x
25	RA	0	0	
26	RA	0	0	x
27	RA	0	0	x
28	RA	0	0	
29	RA	0	0	x
30	RA	0	0	x
31	RA	0	2	x
32	RA	0	0	
33	RA	0	0	x
34	RA	0	0	
35	RA	0	0	
36	RA	0	0	
37	SLE	1	2	x
38	SLE	1	2	
39	SLE	1	1	
40	SLE	1	1	x
41	SLE	1	2	x
42	SLE	1	1	x
43	SLE	1	1	
44	SLE	1	1	x
45	SLE	1	2	x
46	SLE	1	2	x
47	SLE	1	1	x
48	SLE	1	1	x
49	SLE	1	1	x
50	SLE	1	1	
51	SLE	1	1	x
52	SLE	1	1	x

Table 1: Samples used in array and PCR expression profiling experiments

Patient	PrimaryDx	Dx1	Dx2	PCR
53	SLE	1	2	x
54	SLE	1	1	
55	SLE	1	2	x
56	SLE	1	2	x
57	SLE	1	1	
58	SLE	1	1	

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
515	NM_031157	13	Hs.249495	heterogeneous nuclear ribonucleoprotein A1 (HNRPA1), transcript variant 2, mRNA /cds=(104,1222)	0.0909	down
516	D23660	14	Hs.334822	Homo sapiens, Similar to ribosomal protein L4, clone MGC:2966 IMAGE:3139805, mRNA, complete cds /cds=(1616,2617)	0.0909	down
519	BE550944	17	Hs.61426	602329933F1 cDNA	0.0909	down
520	L13385	18	Hs.77318	Miller-Dieker lissencephaly protein (LIS1)	0.0909	down
521	AF315591	19	Hs.6151	pumilio (Drosophila) homolog 2 (PUM2)	0.0909	down
522	AK025620	20	Hs.5985	cDNA: FLJ21967 fis, clone HEP05652, highly similar to AF131831 clone 25186 mRNA sequence /cds=UNKNOWN	0.0909	down
523	AK026747	21	Hs.12969	cDNA: FLJ23094 fis, clone LNG07379	0.0909	down
524	NM_001731	22	Hs.77054	B-cell translocation gene 1, anti-proliferative (BTG1), mRNA /cds=(308,823)	0.0909	down
525	NM_004281	23	Hs.15259	BCL2-associated athanogene 3 (BAG3), mRNA /cds=(306,2033)	0.0909	down
526	XM_008738	24	Hs.79241	B-cell CLL/lymphoma 2 (BCL2), nuclear gene encoding mitochondrial protein, transcript variant alpha, mRNA /cds=(31,750)	0.0909	down
527	XM_018498	25	Hs.180946	ribosomal protein L5 pseudogene mRNA, complete cds /cds=UNKNOWN	0.0909	down
528	U67093	26	Hs.194382	ataxia telangiectasia (ATM) gene, complete cds /cds=(795,9965)	0.0909	down
529	AJ400717	27	Hs.279860	tumor protein, translationally-controlled 1 (TPT1), mRNA /cds=(94,612)	0.0909	down
530	NM_003133	28	Hs.75975	signal recognition particle 9kD (SRP9), mRNA /cds=(106,366)	0.1042	down
531	NM_004261	29	Hs.90606	15 kDa selenoprotein (SEP15), mRNA /cds=(4,492)	0.1042	down
532	NM_002300	30	Hs.234489	Homo sapiens, lactate dehydrogenase B, clone MGC:3600 IMAGE:3028947, mRNA, complete cds /cds=(1745,2749)	0.1071	down
533	NM_003853	31	Hs.158315	interleukin 18 receptor accessory protein (IL18RAP), mRNA /cds=(483,2282)	0.1071	down
534	X53777	32	Hs.82202	ribosomal protein L17 (RPL17), mRNA /cds=(286,840)	0.1071	down
535	N27575	33	Hs.75613	CD36 antigen (collagen type I receptor, thrombospondin receptor) (CD36), mRNA /cds=(132,1550)	0.1167	down
536	NM_006800	34	Hs.88764	male-specific lethal-3 (Drosophila)-like 1 (MSL3L1), mRNA /cds=(105,1670)	0.1167	down
537	NM_000734	35	Hs.97087	CD3Z antigen, zeta polypeptide (TiT3 complex) (CD3Z), mRNA /cds=(178,669)	0.141	down
538	NM_003756	36	Hs.58189	eukaryotic translation initiation factor 3, subunit 3 (gamma, 40kD) (EIF3S3), mRNA /cds=(5,1063)	0.141	down
539	NM_021950	37	Hs.89751	CD20 antigen	0.141	down
540	AK021632	38	Hs.11571	cDNA FLJ11570 fis, clone HEMBA1003309 /cds=UNKNOWN	0.141	down

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
541	AK025583	39	Hs.82845	cDNA: FLJ21930 fis, clone HEP04301, highly similar to HSU90916 clone 23815 mRNA sequence /cds=UNKNOWN	0.141	down
542	NM_000661	40	Hs.157850	Homo sapiens, clone MGC:15545 IMAGE:3050745, mRNA, complete cds /cds=(1045,1623)	0.141	down
543	NM_001057	41	Hs.161305	tachykinin receptor 2 (TACR2), mRNA /cds=(0,1196)	0.141	down
544	X60656	42	Hs.275959	eukaryotic translation elongation factor 1 beta 2 (EEF1B2), mRNA /cds=(235,912)	0.141	down
545	NM_004779	43	Hs.26703	CNOT8 CCR4-NOT transcription complex, subunit 8	0.1628	down
546	X58529	44	Hs.302063	rearranged immunoglobulin mRNA for mu heavy chain enhancer and constant region /cds=UNKNOWN	0.1628	down
547	NM_016091	45	Hs.119503	HSPC025 (HSPC025), mRNA /cds=(33,1727)	0.1705	down
548	NM_001006	46	Hs.77039	ribosomal protein S3A (RPS3A), mRNA /cds=(36,8	0.1739	down
549	NM_001568	47	Hs.106673	eukaryotic translation initiation factor 3, subunit 6 (48kD) (EIF3S6), mRNA /cds=(22,1359)	0.1739	down
550	BC001854	48	Hs.77502	, methionine adenosyltransferase II, alpha, c	0.193	down
551	NM_000983	49	Hs.326249	ribosomal protein L22 (RPL22), mRNA /cds=(51,437)	0.193	down
552	NM_001006	50	Hs.155101	mRNA for KIAA1578 protein, partial cds /cds=(0,3608)	0.193	down
553	NM_001403	51	Hs.274466	eukaryotic translation elongation factor 1 alpha 1-like 14 (EEF1A1L14), mRNA /cds=(620,1816)	0.193	down
554	NM_002796	52	Hs.89545	proteasome (prosome, macropain) subunit, beta type, 4 (PSMB4), mRNA /cds=(23,817)	0.193	down
555	NM_016304	53	Hs.284162	60S ribosomal protein L30 isolog (LOC51187), mRNA /cds=(143,634)	0.193	down
556	NM_017918	54	Hs.234149	hypothetical protein FLJ20647 (FLJ20647), mRNA /cds=(90,836)	0.193	down
557	AA788623	55	Hs.332583	yc77a06.s1 cDNA, 3' end /clone=IMAGE:21844 /clone_end=3'	0.193	down
558	NM_001961	56	Hs.75309	eukaryotic translation elongation factor 2 (EEF2), mRNA /cds=(0,2576)	0.193	down
559	AK026309	57	Hs.12436	cDNA: FLJ22656 fis, clone HSI07655 /cds=UNKNOWN	0.193	down
560	AK026528	58	Hs.334807	Homo sapiens, ribosomal protein L30, clone MGC:2797, mRNA, complete cds /cds=(29,376)	0.1949	down
561	BC002971	59	Hs.1600	Homo sapiens, clone IMAGE:3543711, mRNA, partial cds /cds=(0,1620)	0.1949	down
562	U01923	60	Hs.278857	heterogeneous nuclear ribonucleoprotein H2 (H') (HNRPH2), mRNA /cds=(78,1427)	0.2131	down
563	U61267	61	Hs.30035	putative splice factor transformer2-beta mRN	0.2133	down
564	X14356	62	Hs.77424	high affinity Fc receptor (FcRI) /cds=(36,116	0.2133	down
565	AF267856	63	Hs.8084	HT033 mRNA, complete cds /cds=(203,931)	0.2133	down
566	AK025306	64	Hs.2083	cDNA: FLJ21653 fis, clone COL08586, highly similar to HUMKINCDC protein kinase mRNA /cds=UNKNOWN	0.2133	down

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
567	AL162068	65	Hs.302649	HSP22-like protein interacting protein (LOC64165), mRNA /cds=(0,155)	0.2133	down
568	NM_004768	66	Hs.11482	splicing factor, arginine/serine-rich 11 (SFRS11), mRNA /cds=(83,1537)	0.2133	
569	NM_005594	67	Hs.158164	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) (TAP1), mRNA /cds=(30,2456)	0.2133	down
570	AI440234	68	Hs.9614	Nucleophosmin (probe bad, mutations, wrong clone used) (nucleolar phosphoprotein B23, numatrin)	0.2133	down
571	AW194379	69	Hs.203755	xm08h07.x1 cDNA, 3' end /clone=IMAGE:2683645 /clone_end=3'	0.2133	down
572	NM_005826	70	Hs.15265	heterogeneous nuclear ribonucleoprotein R (HNRPR), mRNA /cds=(90,1991)	0.2133	down
573	AI568695	71	Hs.75969	proline-rich protein with nuclear targeting signal (B4-2), mRNA /cds=(113,1096)	0.2133	down
574	AL110225	72	Hs.89434	drebrin 1 (DBN1), mRNA /cds=(97,2046)	0.2171	down
575	AL110151	73	Hs.128797	mRNA; cDNA DKFZp586D0824 (from clone DKFZp586D0824); partial cds /cds=(0,1080)	0.2403	down
576	NM_006495	74	Hs.5509	ecotropic viral integration site 2B (EVI2B), mRNA /cds=(0,1346)	0.2628	down
577	M74002	75	Hs.11482	splicing factor, arginine/serine-rich 11 (SFRS11), mRNA /cds=(83,1537)	0.2759	down
578	AK002173	76	Hs.5518	cDNA FLJ11311 fis, clone PLACE1010102 /cds=UNKNOWN	0.2759	down
579	AK024976	77	Hs.323378	coated vesicle membrane protein (RNP24), mRNA /cds=(27,632)	0.2759	down
580	BC000967	78	Hs.195870	chronic myelogenous leukemia tumor antigen 66 mRNA, complete cds, alternatively spliced /cds=(232,1983)	0.2759	down
581	NM_016312	79	Hs.334811	Npw38-binding protein NpwBP (LOC51729), mRNA /cds=(143,2068)	0.2759	down
582	X57347	80	Hs.74405	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide (YWHAQ), mRNA /cds=(100,837)	0.2759	down
583	BG424974	81	Hs.292457	Homo sapiens, clone MGC:16362 IMAGE:3927795, mRNA, complete cds /cds=(498,635)	0.276	down
584	U89387	82	Hs.194638	polymerase (RNA) II (DNA directed) polypeptide D (POLR2D), mRNA /cds=(30,458)	0.2784	down
585	AB034205	83	Hs.278670	Acid-inducible phosphoprotein	0.3	down
586	XM_008062	84	Hs.17279	tyrosylprotein sulfotransferase 1 (TPST1), mRNA /cds=(81,1193)	0.3	down
587	NM_016099	85	Hs.7953	HSPC041 protein (LOC51125), mRNA /cds=(141,455)	0.3022	down
588	NM_022898	86	Hs.57987	B-cell lymphoma/leukaemia 11B (BCL11B), mRNA /cds=(267,2738)	0.3533	down
589	NM_006759	87	Hs.77837	UDP-glucose pyrophosphorylase 2 (UGP2), mRNA /cds=(84,1610)	0.367	down
590	AF079566	88	Hs.4311	SUMO-1 activating enzyme subunit 2 (UBA2), mRNA /cds=(25,1947)	0.3798	down
591	NM_001024	89	Hs.182979	cDNA: FLJ22838 fis, clone KAIA4494, highly similar to HUML12A ribosomal protein L12 mRNA /cds=UNKNOWN	0.3798	down
592	NM_017761	90	Hs.7862	hypothetical protein FLJ20312 (FLJ20312), mRNA /cds=(133,552)	0.3798	down

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
593	U15085	91	Hs.1162	major histocompatibility complex, class II, DM beta (HLA-DMB), mRNA /cds=(233,1024)	0.3798	down
594	AW572538	92	Hs.42915	ARP2 (actin-related protein 2, yeast) homolog (ACTR2), mRNA /cds=(74,1258)	0.3798	down
595	AK025557	93	Hs.110771	cDNA: FLJ21904 fis, clone HEP03585 /cds=UNKNOWN	0.3798	down
596	NM_003854	94	Hs.102865	interleukin 1 receptor-like 2 (IL1RL2), mRNA /cds=(134,1822)	0.3798	down
597	AF116679	95	Hs.288036	tRNA isopentenylpyrophosphate transferase (IPT), mRNA /cds=(60,1040)	0.38	down
598	AF148537	96	Hs.65450	reticulon 4a mRNA, complete cds /cds=(141,3719)	0.3857	down
599	NM_017892	97	Hs.107213	hypothetical protein FLJ20585 (FLJ20585), mRNA /cds=(99,746)	0.3972	down
600	NM_000967	98	Hs.119598	ribosomal protein L3 (RPL3), mRNA /cds=(6,1217)	0.4174	down
601	NM_000971	99	Hs.153	ribosomal protein L7 (RPL7), mRNA /cds=(10,756)	0.4174	down
602	AF012872	100	Hs.334874	phosphatidylinositol 4-kinase 230 (pi4K230) mRNA, complete cds /cds=(0,6134)	0.4174	down
603	BC004900	101	Hs.151242	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1 (SERPING1), mRNA /cds=(60,1562)	0.4174	down
604	NM_002298	102	Hs.76506	lymphocyte cytosolic protein 1 (L-plastin) (LCP1), mRNA /cds=(173,2056)	0.4174	down
605	X59405	103	Hs.83532	H.sapiens, gene for Membrane cofactor protein /cds=UNKNOWN	0.4174	down
606	AL049935	104	Hs.301763	mRNA; cDNA DKFZp564O1116 (from clone DKFZp564O1116) /cds=UNKNOWN	0.4174	down
607	NM_017860	105	Hs.79457	hypothetical protein FLJ20519 (FLJ20519), mRNA /cds=(74,604)	0.4181	down
608	J04142	106	Hs.1799	CD1D antigen, d polypeptide (CD1D), mRNA /cds=(164,1171)	0.4231	down
609	NM_016127	107	Hs.279921	HSPC035 protein (LOC51669), mRNA /cds=(16,1035)	0.4622	down
610	AK023379	108	Hs.155160	Homo sapiens, Similar to splicing factor, arginine/serine-rich 2 (SC-35)	0.4798	down
611	L11284	109	Hs.3446	mitogen-activated protein kinase kinase 1 (MAP2K1), mRNA /cds=(72,1253)	0.4798	down
612	NM_002710	110	Hs.79081	protein phosphatase 1, catalytic subunit, gamma isoform (PPP1CC), mRNA /cds=(154,1125)	0.4798	down
613	NM_004380	111	Hs.23598	CREB binding protein (Rubinstein-Taybi syndrome) (CREBBP), mRNA /cds=(198,7526)	0.4798	down
614	AW028193	112	Hs.135872	wv61h08.x1 cDNA, 3' end /clone=IMAGE:2534079 /clone_end=3'	0.4798	down
615	NM_001436	113	Hs.99853	fibrillarin (FBL), mRNA /cds=(59,1024)	0.484	down
616	AB007916	114	Hs.214646	mRNA for KIAA0447 protein, partial cds /cds=(233,1633)	0.5	down
617	AL137681	115	Hs.173912	eukaryotic translation initiation factor 4A, isoform 2 (EIF4A2), mRNA /cds=(15,1238)	0.5	down
618	BC003090	116	Hs.75193	COP9 homolog (COP9), mRNA /cds=(49,678)	0.5	down
619	U15173	117	Hs.155596	BCL2/adenovirus E1B 19kD-interacting protein 2 (BNIP2), mRNA /cds=(211,1155)	0.5	down

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
620	NM_014210	118	Hs.70499	ecotropic viral integration site 2A (EVI2A), mRNA /cds=(219,917)	0.5301	down
621	NM_001011	119	Hs.301547	ribosomal protein S7 (RPS7), mRNA /cds=(81,665)	0.5331	down
622	U07802	120	Hs.78909	Tis11d gene, complete cds /cds=(291,1739)	0.5331	down
623	AI817153	121	Hs.86693	EST380760 cDNA	0.5331	down
624	NM_006791	122	Hs.6353	MORF-related gene 15 (MRG15), mRNA /cds=(131,1102)	0.56	down
625	NM_004500	123	Hs.182447	heterogeneous nuclear ribonucleoprotein C (C1/C2) (HNRPC), transcript variant 1, mRNA /cds=(191,1102)	0.5616	down
626	M16660	124	Hs.318720	Homo sapiens, clone MGC:12387 IMAGE:3933019, mRNA, complete cds /cds=(63,863)	0.588	down
627	NM_001000	125	Hs.300141	cDNA FLJ14163 fis, clone NT2RP1000409 /cds=UNKNOWN	0.5909	down
628	BC008737	126	Hs.164280	Homo sapiens, Similar to solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5, clone MGC:3042 IMAGE:3342722, mRNA, complete cds /cds=(88,984)	0.5938	down
629	BE222392	127	Hs.79914	lumican (LUM), mRNA /cds=(84,1100)	0.6062	down
630	BC010112	128	Hs.79037	Homo sapiens, heat shock 60kD protein 1 (chaperonin), clone MGC:19755 IMAGE:3630225, mRNA, complete cds /cds=(1705,3396)	0.6062	down
631	AK025586	129	Hs.27268	cDNA: FLJ21933 fis, clone HEP04337 /cds=UNKNOWN	0.6224	down
632	NM_015057	130	Hs.151411	KIAA0916 protein (KIAA0916), mRNA /cds=(146,14071)	0.6351	down
633	U10550	131	Hs.79022	GTP-binding protein overexpressed in skeletal muscle (GEM), mRNA /cds=(213,1103)	0.6433	down
634	NM_000986	132	Hs.184582	DPP7 alveolar r	0.6487	down
635	NM_000993	133	Hs.184014	ribosomal protein L31 (RPL31), mRNA /cds=(7,384)	0.6487	down
636	NM_001688	134	Hs.81634	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1 (ATP5F1), mRNA /cds=(32,802)	0.6487	down
637	AI356505	135	Hs.228874	qz22b04.x1 cDNA, 3' end /clone=IMAGE:2027599 /clone_end=3'	0.6487	down
638	AF119850	136	Hs.2186	Homo sapiens, eukaryotic translation elongation factor 1 gamma, clone MGC:4501 IMAGE:2964623, mRNA, complete cds /cds=(2278,3231)	0.6487	down
639	AF132197	137	Hs.301824	hypothetical protein PRO1331 (PRO1331), mRNA /cds=(422,616)	0.6667	down
640	NM_006925	138	Hs.166975	splicing factor, arginine/serine-rich 5 (SFRS5), mRNA /cds=(218,541)	0.6667	down
641	NM_002001	139	Hs.897	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide (FCER1A), mRNA /cds=(106,879)	0.6667	down
513	BC036402	11	NA	116C9	0.6691	up
642	W00466	140	Hs.44189	yz99f01.s1 cDNA, 3' end /clone=IMAGE:291193 /clone_end=3'	0.6691	up

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
643	D17042	141	Hs.50651	Janus kinase 1 (a protein tyrosine kinase) (JAK1), mRNA /cds=(75,3503)	0.6867	down
644	NM_003380	142	Hs.297753	vimentin (VIM), mRNA /cds=(122,1522)	0.6867	down
645	NM_016824	143	Hs.324470	adducin 3 (gamma) (ADD3), transcript variant 1, mRNA /cds=(31,2151)	0.6867	down
646	AI581383	144	Hs.327922	to71c02.x1 cDNA, 3' end /clone=IMAGE:2183714 /clone_end=3'	0.6867	down
647	BC005913	145	Hs.1074	surfactant, pulmonary-associated protein C (SFTPC), mRNA /cds=(27,620)	0.6994	down
648	NM_004811	146	Hs.49587	leupaxin (LPXN), mRNA /cds=(93,1253)	0.6994	down
649	AL357536	147	Hs.3576	Homo sapiens, Similar to RIKEN cDNA 5730494N06 gene, clone MGC:13348 IMAGE:4132400, mRNA, complete cds /cds=(132,494)	0.7029	down
650	NM_022570	148	Hs.161786	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 12 (CLECSF12), mRNA /cds=(71,676)	0.7029	down
651	NM_004396	149	Hs.76053	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68kD) (DDX5), mRNA /cds=(170,2014)	0.7151	down
652	AK026372	150	Hs.143631	cDNA: FLJ22719 fis, clone HSI14307 /cds=UNKNOWN	0.7225	down
653	XM_012059	151	Hs.154938	hypothetical protein MDS025 (MDS025), mRNA /cds=(5,769)	0.7299	down
654	XM_011914	152	Hs.180450	ribosomal protein S24 (RPS24), transcript variant 1, mRNA /cds=(37,429)	0.7301	down
655	NM_020414	153	Hs.286233	sperm autoantigenic protein 17 (SPA17), mRNA /cds=(1210,1665)	0.7301	down
656	S73591	154	Hs.179526	upregulated by 1,25-dihydroxyvitamin D-3 (VDUP1), mRNA /cds=(221,1396)	0.7373	down
657	J00194	155	Hs.76807	major histocompatibility complex, class II, DR alpha (HLA-DRA), mRNA /cds=(26,790)	0.7989	down
658	AK021715	156	Hs.271541	cDNA FLJ11653 fis, clone HEMBA1004538 /cds=UNKNOWN	0.7989	down
659	AK027187	157	Hs.289071	cDNA: FLJ22245 fis, clone HRC02612 /cds=UNKNOWN	0.7989	down
660	AL117595	158	Hs.4055	mRNA; cDNA DKFZp564C2063 (from clone DKFZp564C2063) /cds=UNKNOWN	0.7989	down
661	NM_002823	159	Hs.250655	prothymosin, alpha (gene sequence 28) (PTMA), mRNA /cds=(155,487)	0.7989	down
662	NM_004327	160	Hs.234799	breakpoint cluster region (BCR), transcript variant 1, mRNA /cds=(488,4303)	0.7989	down
663	NM_017830	161	Hs.132071	ovarian carcinoma immunoreactive antigen (OCIA), mRNA /cds=(167,904)	0.7989	down
664	X06557	162	Hs.2014	mRNA for T-cell receptor delta /cds=UNKNOWN	0.7989	down
665	AI146787	163	Hs.156601	qb83f02.x1 cDNA, 3' end /clone=IMAGE:1706715 /clone_end=3'	0.7989	down
666	AI568771	164	Hs.327876	th15h04.x1 cDNA, 3' end /clone=IMAGE:2118391 /clone_end=3'	0.7989	down
667	AW195119	165	Hs.253151	xn66b07.x1 cDNA, 3' end /clone=IMAGE:2699413 /clone_end=3'	0.7989	down
668	NM_016316	166	Hs.110347	REV1 (yeast homolog)- like (REV1L), mRNA /cds=(212,3967)	0.8132	down

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
669	AV724531	167	Hs.76728	602570065F1 cDNA, 5' end /clone=IMAGE:4694321 /clone_end=5'	0.8594	down
670	AK002059	168	Hs.92918	hypothetical protein (BM-009), mRNA /cds=(385,1047)	0.8594	down
671	NM_001503	169	Hs.272529	glycosylphosphatidylinositol specific phospholipase D1 (GPLD1), mRNA /cds=(32,2557)	0.8653	down
672	AA251806	170	Hs.177712	zs09c03.s1 cDNA, 3' end /clone=IMAGE:684676 /clone_end=3'	0.8734	up
517	NM_006276	15	Hs.184167	splicing factor, arginine/serine-rich 7 (35kD) (SFRS7) mRNA /cds=(105,490)	0.8883	down
673	NM_004315	171	Hs.75811	N-acylsphingosine amidohydrolase (acid ceramidase) (ASAH), mRNA /cds=(17,1204)	0.8883	down
674	NM_004371	172	Hs.75887	coatomer protein complex, subunit alpha (COPA), mRNA /cds=(466,4140)	0.8883	down
675	AF054284	173	Hs.334826	splicing factor 3b, subunit 1, 155kD (SF3B1), mRNA /cds=(0,3914)	0.8889	down
676	BE613237	174	Hs.146381	RNA binding motif protein, X chromosome (RBMX), mRNA /cds=(11,1186)	0.9257	down
677	NM_003367	175	Hs.93649	upstream transcription factor 2, c-fos intera	0.9542	up
678	AB014522	176	Hs.11238	mRNA for KIAA0622 protein, partial cds /cds=(0,3869)	0.9542	up
679	AW137104	177	Hs.8121	Notch (Drosophila) homolog 2 (NOTCH2), mRNA /cds=(12,7427)	0.9542	up
680	BF897042	178	Hs.120219	FLJ32028 hypothetical protein FLJ32028	0.9542	up
681	BC002900	179	Hs.181309	proteasome (prosome, macropain) subunit, alpha type, 2 (PSMA2), mRNA /cds=(0,704)	0.9563	down
682	AL578975	180	Hs.5057	AL578975 cDNA /clone=CS0DK012YN01-(3-prime)	0.9563	down
683	NM_000988	181	Hs.111611	ribosomal protein L27 (RPL27), mRNA /cds=(17,427)	0.9662	down
684	NM_003769	182	Hs.77608	splicing factor, arginine/serine-rich 9 (SFRS9), mRNA /cds=(52,717)	1.0167	down
685	U94855	183	Hs.7811	eukaryotic translation initiation factor 3, subunit 5 (epsilon, 47kD) (EIF3S5), mRNA /cds=(6,1079)	1.0167	down
686	AV749844	184	Hs.26670	PAC clone RP3-515N1 from 22q11.2-q22 /cds=(0,791)	1.0236	down
687	BC003352	185	Hs.326456	hypothetical protein FLJ20030 (FLJ20030), mRNA /cds=(1,1239)	1.0236	down
688	AU135154	186	Hs.172028	a disintegrin and metalloproteinase domain 10 (ADAM10), mRNA /cds=(469,2715)	1.0327	down
689	AF208850	187	Hs.82911	BM-008 mRNA, complete cds /cds=(341,844)	1.0822	down
690	D29805	188	Hs.198248	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1 (B4GALT1), mRNA /cds=(72,1268)	1.0822	down
691	NM_006098	189	Hs.5662	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1 (GNB2L1), mRNA /cds=(95,1048)	1.0822	down
692	NM_001755	190	Hs.179881	core-binding factor, beta subunit (CBFB), transcript variant 2, mRNA /cds=(11,559)	1.0905	down
693	NM_007355	191	Hs.74335	heat shock 90kD protein 1, beta (HSPCB), mRNA /cds=(0,2174)	1.0905	down

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
694	W47229	192	Hs.173334	ELL-RELATED RNA POLYMERASE II, ELONGATION FACTOR (ELL2), mRNA /cds=(0,1922)	1.1	
695	X51345	193	Hs.198951	jun B proto-oncogene (JUNB), mRNA /cds=(253,1296)	1.1	
506	NM_006417	4	Hs.82316	interferon-induced, hepatitis C-associated microtubular aggregate protein (44kD) (MTAP44), mRNA /cds=(0,1334)	1.1077	up
696	AI364677	194	Hs.368853	ESTs	1.1077	up
697	AI380594	195	Hs.231261	tf95h06.x1 cDNA, 3' end /clone=IMAGE:2107067 /clone_end=3'	1.1077	up
698	NM_001641	196	Hs.73722	APEX nuclease (multifunctional DNA repair enzyme) (APEX), mRNA /cds=(205,1161)	1.1138	down
699	NM_002786	197	Hs.82159	proteasome (prosome, macropain) subunit, alpha type, 1 (PSMA1), mRNA /cds=(105,896)	1.1138	down
700	J02621	198	Hs.251064	high-mobility group (nonhistone chromosomal) protein 14 (HMG14), mRNA /cds=(150,452)	1.115	down
701	NM_019111	199	Hs.76807	major histocompatibility complex, class II, DR alpha (HLA-DRA), mRNA /cds=(26,790)	1.115	down
702	AF248966	200	Hs.183434	ATPase, H+ transporting, lysosomal (vacuolar proton pump) membrane sector associated protein M8-9 (APT6M8-9)	1.1336	down
703	D31767	201	Hs.75416	DAZ associated protein 2 (DAZAP2), mRNA /cds=(69,575)	1.1336	down
704	NM_006839	202	Hs.78504	inner membrane protein, mitochondrial (mitofilin) (IMMT), mRNA /cds=(92,2368)	1.1336	down
705	AI581732	203	Hs.229918	ar74f03.x1 cDNA, 3' end /clone=IMAGE:2128349 /clone_end=3'	1.1336	down
706	NM_019059	204	Hs.274248	hypothetical protein FLJ20758 (FLJ20758), mRNA /cds=(464,1306)	1.1438	down
707	NM_001033	205	Hs.2934	ribonucleotide reductase M1 polypeptide (RRM1), mRNA /cds=(187,2565)	1.1525	down
708	NM_002719	206	Hs.171734	protein phosphatase 2, regulatory subunit B (B56), gamma isoform (PPP2R5C), mRNA /cds=(88,1632)	1.1525	down
709	NM_003791	207	Hs.75890	membrane-bound transcription factor protease, site 1 (MBTPS1), mRNA /cds=(496,3654)	1.1772	down
710	NM_001105	208	Hs.150402	activin A receptor, type I (ACVR1), mRNA /cds=(340,1869)	1.1833	down
711	BG179517	209	Hs.99093	chromosome 19, cosmid R28379 /cds=(0,633)	1.1833	down
712	BF940103	210	Hs.26136	hypothetical protein MGC14156 (MGC14156), mRNA /cds=(82,426)	1.1833	down
713	AF061736	211	Hs.169895	ubiquitin-conjugating enzyme E2L 6 (UBE2L6), mRNA /cds=(47,508)	1.6	
714	AK023680	212	Hs.17448	cDNA FLJ13618 fis, clone PLACE1010925 /cds=UNKNOWN	1.6	
715	NM_001295	213	Hs.301921	chemokine (C-C motif) receptor 1 (CCR1), mRNA /cds=(62,1129)	1.6	
716	NM_003811	214	Hs.1524	tumor necrosis factor (ligand) superfamily, member 9 (TNFSF9), mRNA /cds=(3,767)	1.6	
717	X02812	215	Hs.1103	transforming growth factor, beta 1 (TGFB1), mRNA /cds=(841,2016)	1.6	
718	NM_002205	216	Hs.149609	integrin, alpha 5 (fibronectin receptor, alpha polypeptide) (ITGA5), mRNA /cds=(23,3172)	1.6	

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
719	AI818777	217	Hs.229990	wl11f10.x1 cDNA, 3' end /clone=IMAGE:2424619 /clone_end=3'	1.6	
720	NM_005892	218	Hs.100217	formin-like (FMNL), mRNA /cds=(39,1430)	1.6	
721	M26252	219	Hs.198281	pyruvate kinase, muscle (PKM2), mRNA /cds=(109,1704)	1.6	
722	AB002377	220	Hs.32556	mRNA for KIAA0379 protein, partial cds /cds=(0,3180)	1.6324	up
723	AI381586	221	Hs.87908	Snf2-related CBP activator protein (SRCAP), mRNA /cds=(210,9125)	1.6662	up
724	BG760189	222	Hs.37617	602144947F1 cDNA, 5' end /clone=IMAGE:4308683 /clone_end=5'	2.0136	up
725	NM_006913	223	Hs.216354	ring finger protein 5 (RNF5), mRNA /cds=(0,542)	2.0853	up
726	AF189011	224	Hs.49163	ribonuclease III (RN3) mRNA, complete cds /cds=(245,4369)	2.246	up
727	AK022834	225	Hs.58488	catenin (cadherin-associated protein), alpha-like 1 (CTNNAL1), mRNA /cds=(43,2247)	2.3244	up
728	NM_002878	226	Hs.125244	RAD51 (S. cerevisiae)-like 3 (RAD51L3), mRNA /cds=(124,993)	2.3244	up
729	BF899464	227	NA	IL5-MT0211-011200-317-f03 MT0211 cDNA, mRNA sequence	2.3244	up
730	AW452510	228	Hs.300479	UI-H-BW1-ame-a-12-0-UI.s1 cDNA, 3' end /clone=IMAGE:3069598 /clone_end=3'	2.3244	up
731	NM_005508	229	Hs.184926	chemokine (C-C motif) receptor 4 (CCR4), mRNA /cds=(182,1264)	2.3244	up
732	X16354	230	Hs.50964	mRNA for transmembrane carcinoembryonic antigen BGPa (formerly TM1-CEA) /cds=(72,1652)	2.468	up
733	AA581115	231	Hs.291129	oe10d02.s1 cDNA /clone=IMAGE:1385475	2.468	up
734	NM_005485	232	Hs.271742	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)-like 3 (ADPRTL3), mRNA /cds=(246,1847)	2.468	up
735	NM_005816	233	Hs.142023	T cell activation, increased late expression (TACTILE), mRNA /cds=(928,2637)	2.468	up
736	BG033294	234	Hs.118787	transforming growth factor, beta-induced, 68kD (TGFBI), mRNA /cds=(47,2098)	2.468	up
737	K01566	235	Hs.69771	B-factor, properdin	2.468	up
738	T25714	236	Hs.330530	ESTDIR309 cDNA, 3' end /clone=CDDIRX9 /clone_end=3'	2.468	up
739	NM_022873	237	Hs.265827	interferon, alpha-inducible protein (clone IFI-6-16) (G1P3), transcript variant 3, mRNA /cds=(107,523)	2.468	up
740	X99699	238	Hs.139262	XIAP associated factor-1 (HSXIAPAF1), mRNA /cds=(0,953)	2.67	
741	AF067519	239	Hs.307357	PITSLRE protein kinase beta SV1 isoform (CDC2L2) mRNA, complete cds /cds=(79,2412)	2.7771	up
742	BG387694	240	Hs.170980	cell cycle progression 2 protein (CPR2), mRNA /cds=(126,1691)	2.7771	up
743	AF104032	241	Hs.184601	L-type amino acid transporter subunit LAT1 mRNA, complete cds /cds=(66,1589)	2.7771	up
744	NM_012177	242	Hs.272027	F-box only protein 5 (FBXO5), mRNA /cds=(61,1404)	2.7771	up
745	AL042370	243	Hs.79709	phosphotidylinositol transfer protein (PITPN), mRNA /cds=(216,1028)	2.9579	up

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
746	BC009469	244	Hs.287797	mRNA for FLJ00043 protein, partial cds /cds=(0,4248)	2.9579	up
747	AA319163	245	Hs.424299	RPLP1 ;germinal	2.9579	up
748	AI393970	246	Hs.76239	hypothetical protein FLJ20608 (FLJ20608), mRNA /cds=(81,680)	2.9579	up
749	NM_014481	247	Hs.154149	Homo sapiens, apurinic/apyrimidinic endonuclease(APEX nuclease)-like 2 protein, clone MGC:1418 IMAGE:3139156, mRNA, complete cds /cds=(38,1594)	2.9579	up
750	NM_017774	248	Hs.306668	cDNA FLJ14089 fis, clone MAMMA1000257 /cds=UNKNOWN	2.9579	up
751	NM_017859	249	Hs.39850	hypothetical protein FLJ20517 (FLJ20517), mRNA /cds=(44,1690)	2.9579	up
752	R44202	250	Hs.240013	mRNA; cDNA DKFZp547A166 (from clone DKFZp547A166) /cds=UNKNOWN	2.9579	up
753	NM_002904	251	Hs.106061	RD RNA-binding protein (RDBP), mRNA /cds=(108,1250)	2.9579	up
754	AL133642	252	Hs.241471	mRNA; cDNA DKFZp586G1721 (from clone DKFZp586G1721); partial cds /cds=(0,669)	3.09	up
755	AF160973	253	Hs.258503	P53 inducible protein	3.0908	up
756	NM_001972	254	Hs.99863	elastase 2, neutrophil (ELA2),	3.0908	up
757	AA282774	255	NA	cDNA clone IMAGE:713136 5'	3.0908	up
758	AB000115	256	Hs.75470	hypothetical protein, expressed in osteoblast (GS3686), mRNA /cds=(241,1482)	3.0908	up
759	AJ277247	257	Hs.287369	interleukin 22 (IL22), mRNA /cds=(71,610)	3.0908	up
760	D38081	258	Hs.89887	thromboxane A2 receptor (TBXA2R), mRNA /cds=(991,2022)	3.0908	up
761	NM_001250	259	Hs.25648	tumor necrosis factor receptor superfamily, member 5 (TNFRSF5), mRNA /cds=(47,880)	3.0908	up
762	AI524266	260	Hs.230874	th11g12.x1 cDNA, 3' end /clone=IMAGE:2118022 /clone_end=3'	3.0908	up
763	AL573787	261	Hs.21732	AL573787 cDNA /clone=CS0DI055YM17-(3-prime)	3.0908	up
764	AK001503	262	Hs.265891	cDNA FLJ10641 fis, clone NT2RP2005748 /cds=UNKNOWN	3.0908	up
765	X04430	263	Hs.93913	IFN-beta 2a mRNA for interferon-beta-2, T-cells, macrophages	3.0908	up
766	AF480557	264	NA	142E4	3.0908	up
767	AL550229	265	Hs.271599	cDNA FLJ12347 fis, clone MAMMA1002298 /cds=UNKNOWN	3.0908	up
768	AV727063	266	Hs.245798	hypothetical protein DKFZp564I0422 (DKFZP564I0422), mRNA /cds=(510,1196)	3.0908	up
769	NM_000389	267	Hs.179665	cyclin-dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A), mRNA /cds=(75,569)	3.0908	up
770	NM_001761	268	Hs.1973	cyclin F (CCNF), mRNA /cds=(43,2403)	3.0908	up
771	NM_002741	269	Hs.2499	protein kinase C-like 1 (PRKCL1), mRNA /cds=(84,2912)	3.0908	up
772	NM_002880	270	Hs.279474	HSPC070 protein (HSPC070), mRNA /cds=(331,1581)	3.0908	up

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
773	NM_014373	271	Hs.97101	putative G protein-coupled receptor (GPCR150), mRNA /cds=(321,1337)	3.0908	up
774	U53347	272	Hs.183556	solute carrier family 1 (neutral amino acid transporter), member 5 (SLC1A5), mRNA /cds=(590,2215)	3.0908	up
775	W19201	273	Hs.17778	neuropilin 2 (NRP2), mRNA /cds=(0,2780)	3.0908	up
776	W79598	274	Hs.163846	putative N6-DNA-methyltransferase (N6AMT1), mRNA /cds=(29,673)	3.0908	up
777	XM_001939	275	Hs.55468	H4 histone, family 2	3.0908	up
778	AI270476	276	Hs.270341	602307338F1 cDNA, 5' end /clone=IMAGE:4398848 /clone_end=5'	3.0908	up
779	AA992299	277	Hs.129332	ot53b06.s1 cDNA, 3' end /clone=IMAGE:1620467 /clone_end=3'	3.0908	up
780	AF044595	278	Hs.248078	lymphocyte-predominant Hodgkin's disease case #7 immunoglobulin heavy chain gene, variable region	3.0908	up
781	BI091076	279	Hs.127128	ok13e12.s1 cDNA, 3' end /clone=IMAGE:1507726 /clone_end=3'	3.0908	up
782	H13491	280	Hs.303450	yj15f02.r1 cDNA, 5' end /clone=IMAGE:148827 /clone_end=5'	3.0908	up
783	M55420	281	Hs.247930	IgE chain, last 2 exons	3.0908	up
784	NM_014271	282	Hs.241385	interleukin 1 receptor accessory protein-like 1 (IL1RAPL1), mRNA /cds=(510,2600)	3.0908	up
785	AI378091	283	Hs.369056	ESTs	3.0908	up
786	AI381601	284	Hs.159025	td05g03.x1 cDNA, 3' end /clone=IMAGE:2074804 /clone_end=3'	3.0908	up
787	AI634972	285	Hs.319825	602021477F1 cDNA, 5' end /clone=IMAGE:4156915 /clone_end=5'	3.0908	up
788	AW005376	286	Hs.173280	ws94a12.x1 cDNA, 3' end /clone=IMAGE:2505598 /clone_end=3'	3.0908	up
789	AW088500	287	Hs.389655	EST, Weakly similar to A35098 MHC class III histocompatibility antigen HLA-B-associated transcript 3	3.0908	up
790	AW195270	288	Hs.330019	xn67c04.x1 cDNA, 3' end /clone=IMAGE:2699526 /clone_end=3'	3.0908	up
791	AW296797	289	Hs.255579	UI-H-BW0-ajb-e-07-0-UI.s1 cDNA, 3' end /clone=IMAGE:2731117 /clone_end=3'	3.0908	up
792	BF827734	290	Hs.156766	ESTs	3.0908	up
793	M11233	291	Hs.79572	cathepsin D (lysosomal aspartyl protease) (CTSD), mRNA /cds=(2,1240)	3.0908	up
794	AL050218	292	Hs.15020	DNA sequence from clone 51J12 on chromosome 6q26-27.	3.1014	up
795	NM_016063	293	Hs.32826	CGI-130 protein (LOC51020), mRNA /cds=(63,575)	3.1014	up
796	BU678165	294	Hs.377992	479H5, not in ref seq, Rab geranylgeranyltransferase, alpha subunit (RABGGTA),	3.1014	up
797	AL050371	295	Hs.8128	phosphatidylserine decarboxylase (PISD), mRNA /cds=(223,1350)	3.33	up

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
798	NM_152545	296	Hs.335815	62C9, hypothetical protein FLJ31695	3.3318	up
799	XM_007156	297	Hs.159492	sacsin (SACS) gene, complete cds /cds=(76,11565)	3.3318	up
800	NM_014339	298	Hs.129751	interleukin 17 receptor (IL17R), mRNA /cds=(32,2632)	3.3318	up
801	NM_019598	299	Hs.159679	kallikrein 12 (KLK12), mRNA /cds=UNKNOWN	3.3318	up
802	AI081258	300	Hs.134590	oy67c11.x1 cDNA, 3' end /clone=IMAGE:1670900 /clone_end=3'	3.3318	up
803	AW468621	301	Hs.257743	he42e03.x1 cDNA, 3' end /clone=IMAGE:2921692 /clone_end=3'	3.3318	up
804	NM_001873	302	Hs.75360	carboxypeptidase E (CPE), mRNA /cds=(290,1720)	3.369	up
805	NM_032839	303	Hs.11360	hypothetical protein FLJ14784 (FLJ14784), mRNA /cds=(133,1569)	3.369	up
806	X16277	304	Hs.339703	zv26f06.r1 cDNA, 5' end /clone=IMAGE:754787 /clone_end=5'	3.4341	up
807	NM_000395	305	Hs.285401	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage) (CSF2RB), mRNA /cds=(28,2721)	3.4341	
808	NM_013252	306	Hs.126355	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 5 (CLECSF5), mRNA /cds=(197,763)	3.5169	up
809	129F10	307	NA	129F10, chromosome hit	3.7268	up
810	AK024331	308	Hs.287631	cDNA FLJ14269 fis, clone PLACE1003864 /cds=UNKNOWN	3.7268	up
811	NM_000195	309	Hs.83951	Hermansky-Pudlak syndrome (HPS), mRNA /cds=(206,2308)	3.7268	up
812	NM_030756	310	Hs.173638	transcription factor 7-like 2 (T-cell specific, HMG-box) (TCF7L2), mRNA /cds=(307,2097)	3.7268	up
813	M26683	311	Hs.303649	interferon gamma treatment inducible mRNA Monocytes	3.7833	
814	AA214691	312	Hs.111377	LOC286530 hypothetical protein LOC286530	3.7833	up
815	AB049113	313	Hs.82113	dUTP pyrophosphatase (DUT), mRNA /cds=(29,523)	3.7833	up
816	AK026819	314	Hs.20242	hypothetical protein FLJ12788 (FLJ12788), mRNA /cds=(9,866)	3.7833	up
817	L21961	315	Hs.181125	Homo sapiens, clone MGC:12849 IMAGE:4308973, mRNA, complete cds /cds=(24,725)	3.7833	up
818	NM_001278	316	Hs.306440	mRNA; cDNA DKFZp566L084 (from clone DKFZp566L084) /cds=UNKNOWN	3.7833	up
819	NM_002385	317	Hs.69547	myelin basic protein (MBP), mRNA /cds=(10,570)	3.7833	up
820	NM_005121	318	Hs.11861	thyroid hormone receptor-associated protein, 240 kDa subunit (TRAP240), mRNA /cds=(77,6601)	3.7833	up
821	NM_007220	319	Hs.283646	carbonic anhydrase VB, mitochondrial (CA5B), nuclear gene encoding mitochondrial protein, mRNA /cds=(137,1090)	3.7833	up
822	NM_012381	320	Hs.74420	origin recognition complex, subunit 3 (yeast homolog)-like (ORC3L), mRNA /cds=(26,2161)	3.7833	up
823	NM_014225	321	Hs.173902	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform (PPP2R1A), mRNA /cds=(138,1907)	3.7833	up

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
824	BF966028	322	Hs.5324	hypothetical protein (CL25022), mRNA /cds=(157,1047)	3.7833	up
825	AL157438	323	Hs.66151	mRNA; cDNA DKFZp434A115 (from clone DKFZp434A115) /cds=UNKNOWN	3.7833	up
826	NM_004488	324	Hs.73734	glycoprotein V (platelet) (GP5), mRNA /cds=(270,1952)	3.7833	up
827	NM_006929	325	Hs.153299	DOM-3 (C. elegans) homolog Z (DOM3Z), transcript variant 2, mRNA /cds=(129,1319)	3.7833	up
828	NM_021976	326	Hs.79372	retinoid X receptor, beta (RXRB), mRNA /cds=(179,1780)	3.7833	up
829	T93822	327	Hs.294092	EST375308 cDNA	3.7833	up
830	AI524202	328	Hs.171122	th10d11.x1 cDNA, 3' end /clone=IMAGE:2117877 /clone_end=3'	3.7833	up
831	AI684022	329	Hs.90744	proteasome (prosome, macropain) 26S subunit, non-ATPase, 11 (PSMD11), mRNA /cds=(0,1268)	3.7833	up
832	AW452545	330	Hs.257582	UI-H-BW1-ame-d-12-0-UI.s1 cDNA, 3' end /clone=IMAGE:3069742 /clone_end=3'	3.7833	up
833	NM_153341	331	Hs.64239	DNA sequence from clone RP5-1174N9 on chromosome 1p34.1-35.3. Contains the gene for a novel protein with IBR domain, a (pseudo?) gene for a novel protein similar to MT1E (metallothionein 1E (functional)), ESTs, STSs, GSSs and two putative CpG islands /cd	3.7833	up
834	BF698885	332	Hs.5890	hypothetical protein FLJ23306 (FLJ23306), mRNA /cds=(562,930)	3.7833	up
835	NM_000073	333	Hs.2259	CD3G antigen, gamma polypeptide (TiT3 complex) (CD3G), mRNA /cds=(37,585)	3.8053	up
836	NM_004761	334	Hs.170160	RAB2, member RAS oncogene family-like (RAB2L), mRNA /cds=(0,2333)	3.8053	up
837	NM_015898	335	Hs.104640	HIV-1 inducer of short transcripts binding protein (FBI1), mRNA /cds=(0,1754)	3.8053	up
838	NM_014348	336	Hs.296429	similar to rat integral membrane glycoprotein POM121 (POM121L1), mRNA /cds=(0,1286)	3.8053	up
839	AW500534	337	Hs.145668	fmfc5 cDNA /clone=CR6-21	3.8053	up
840	AA765569	338	Hs.104157	EST380899 cDNA	3.8053	up
841	AI084553	339	Hs.105621	HNC29-1-B1.R cDNA	3.8053	up
842	AI523617	340	Hs.171098	tg95b03.x1 cDNA, 3' end /clone=IMAGE:2116493 /clone_end=3'	3.8053	up
843	AI969716	341	Hs.13034	hv63f09.x1 cDNA, 3' end /clone=IMAGE:3178121 /clone_end=3'	3.8053	up
844	NM_002076	342	Hs.164036	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID) (GNS), mRNA /cds=(87,1745)	3.8053	up
925	BG505271	423	Hs.86437	602411368F1 cDNA, 5' end /clone=IMAGE:4540096 /clone_end=5'	4.1189	up
926	BE965319	424	Hs.286754	601659229R1 cDNA, 3' end /clone=IMAGE:3895783 /clone_end=3'	4.1189	up
927	NM_003264	425	Hs.63668	toll-like receptor 2 (TLR2), mRNA /cds=(129,2483)	4.246	up
928	BU540019	426	NA	485A6, EST	4.2464	up
929	AV719442	427	Hs.256959	AV719442 cDNA, 5' end /clone=GLCBNA01 /clone_end=5'	4.2464	up

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
930	NM_000879	428	Hs.2247	interleukin 5 (colony-stimulating factor, eosinophil) (IL5), mRNA /cds=(44,448)	4.2464	up
931	NM_001916	429	Hs.289271	cytochrome c-1 (CYC1), mRNA /cds=(8,985)	4.2464	up
932	NM_002460	430	Hs.82132	interferon regulatory factor 4 (IRF4), mRNA /cds=(105,1460)	4.2464	up
933	NM_002994	431	Hs.89714	small inducible cytokine subfamily B (Cys-X-Cys), member 5	4.2464	up
934	NM_007015	432	Hs.97932	chondromodulin I precursor (CHM-I), mRNA /cds=(0,1004)	4.2464	up
935	NM_017644	433	Hs.246875	hypothetical protein FLJ20059 (FLJ20059), mRNA /cds=(25,1290)	4.2464	up
936	X57025	434	Hs.85112	IGF-I mRNA for insulin-like growth factor I /cds=(166,627)	4.2464	up
937	BF892532	435	Hs.38664	IL0-MT0152-061100-501-e04 cDNA	4.2464	up
938	BG028577	436	Hs.279009	matrix Gla protein (MGP), mRNA /cds=(46,357)	4.2464	up
939	AF116909	437	Hs.167827	clone HH419 unknown mRNA /cds=(189,593)	4.2464	up
940	AL136842	438	Hs.260024	mRNA; cDNA DKFZp434A0530 (from clone DKFZp434A0530); complete cds /cds=(968,1732)	4.2464	up
941	AW327360	439	Hs.250605	dq02e11.x1 cDNA, 5' end /clone=IMAGE:2846685 /clone_end=5'	4.2464	up
942	AI538420	440	Hs.231292	td06a03.x1 cDNA, 3' end /clone=IMAGE:2074828 /clone_end=3'	4.2464	up
943	AI805144	441	NA	EST	4.2464	up
944	AW064160	442	Hs.279141	SP0594 cDNA, 3' end /clone_end=3'	4.2464	up
945	AW078847	443	Hs.244816	xb18g07.x1 cDNA, 3' end /clone=IMAGE:2576700 /clone_end=3'	4.2464	up
946	AW236252	444	Hs.253747	xn71g08.x1 cDNA, 3' end /clone=IMAGE:2699966 /clone_end=3'	4.2464	up
947	AW297026	445	Hs.255600	UI-H-BW0-ajf-e-06-0-UI.s1 cDNA, 3' end /clone=IMAGE:2731499 /clone_end=3'	4.2464	up
948	NM_016095	446	Hs.108196	HSPC037 protein (LOC51659), mRNA /cds=(78,635)	4.2464	up
949	AK000575	447	Hs.279581	hypothetical protein FLJ20568 (FLJ20568), mRNA /cds=(6,422)	4.2892	up
950	NM_002462	448	Hs.76391	myxovirus (influenza) resistance 1, homolog of murine (interferon-inducible protein p78) (MX1), mRNA /cds=(345,2333)	4.2892	up
951	NM_003841	449	Hs.119684	tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain (TNFRSF10C), mRNA /cds=(29,928)	4.2892	up
952	NM_004834	450	Hs.3628	mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4), mRNA /cds=(79,3576)	4.2892	up
953	NM_013368	451	Hs.169138	RPA-binding trans-activator (RBT1), mRNA /cds=(291,881)	4.2892	up
954	X12451	452	Hs.78056	cathepsin L (CTSL), mRNA /cds=(288,1289)	4.2892	up
955	Y13936	453	Hs.17883	protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform (PPM1G), mRNA /cds=(24,1664)	4.2892	up

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
956	AW190635	454	Hs.15200	EST379783 cDNA	4.2892	up
957	AI378123	455	Hs.327454	tc80e02.x1 cDNA, 3' end /clone=IMAGE:2072474 /clone_end=3'	4.2892	up
958	AJ275405	456	Hs.272362	partial IGVL1 gene for immunoglobulin lambda light chain V region	4.2892	up
959	AA729508	457	Hs.307486	nx54a03.s1 cDNA /clone=IMAGE:1266028	4.2892	up
960	AI865603	458	Hs.341208	wk47g03.x1 cDNA, 3' end /clone=IMAGE:2418580 /clone_end=3'	4.2892	up
961	NM_080612	459	Hs.102630	128F5, GRB2-associated binding protein 3 (GAB3),	4.2892	up
962	NM_014086	460	Hs.6975	PRO1073 protein (PRO1073),	4.3699	up
963	L11695	461	Hs.220	transforming growth factor, beta receptor I (activin A receptor type II-like kinase, 53kD) (TGFBR1), mRNA /cds=(76,1587)	4.3699	up
964	NM_002995	462	Hs.3195	small inducible cytokine subfamily C, member 1 (lymphotactin) (SCYC1), mRNA /cds=(20,364)	4.3699	up
965	BF968963	463	Hs.5064	602490910F1 cDNA, 5' end /clone=IMAGE:4619835 /clone_end=5'	4.3699	up
966	BG286649	464	Hs.323950	zinc finger protein 6 (CMPX1) (ZNF6), mRNA /cds=(1265,3361)	4.3699	up
967	NM_014148	465	Hs.278944	HSPC048 protein (HSPC048), mRNA /cds=(87,419)	4.3699	up
968	BF195579	466	Hs.232257	RST2302 cDNA	4.3699	up
969	BF509758	467	Hs.144265	UI-H-BI4-apg-d-04-0-UI.s1 cDNA, 3' end /clone=IMAGE:3087390 /clone_end=3'	4.3699	up
970	AF118274	468	Hs.128740	DNb-5 mRNA, partial cds /cds=(0,1601)	4.4485	up
971	NM_005082	469	Hs.1579	zinc finger protein 147 (estrogen-responsive finger protein) (ZNF147), mRNA /cds=(39,1931)	4.4485	up
972	AA576947	470	Hs.188886	nm82b04.s1 cDNA, 3' end /clone=IMAGE:1074703 /clone_end=3'	4.4485	up
973	AA628833	471	NA	EST	4.4485	up
974	AI631850	472	Hs.340604	wa36h07.x1 cDNA, 3' end /clone=IMAGE:2300221 /clone_end=3'	4.4485	up
975	AW006867	473	Hs.231987	602320903F1 cDNA, 5' end /clone=IMAGE:4424065 /clone_end=5'	4.4485	up
976	M94046	474	Hs.7647	MYC-associated zinc finger protein (purine-binding transcription factor) (MAZ), mRNA /cds=(91,1584)	4.4977	up
977	AB007861	475	Hs.118047	602971981F1 cDNA, 5' end /clone=IMAGE:5111324 /clone_end=5'	4.5272	up
978	AF061944	476	Hs.432900	PRKWNK1 protein kinase, lysine deficient 1	4.5272	up
979	AL136797	477	Hs.273294	mRNA; cDNA DKFZp434N031 (from clone DKFZp434N031); complete cds /cds=(18,3608)	4.5272	up
980	D42040	478	Hs.75243	bromodomain-containing 2 (BRD2), mRNA /cds=(1701,4106)	4.5272	up
981	AI089359	479	Hs.130232	qb05h03.x1 cDNA, 3' end /clone=IMAGE:1695413 /clone_end=3'	4.5272	up
982	NM_004776	480	Hs.107526	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 5 (B4GALT5), mRNA /cds=(112,1278)	4.5272	up

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
983	NM_020428	481	Hs.105509	cDNA FLJ14613 fis, clone NT2RP1001113, highly similar to CTL2 gene /cds=UNKNOWN	4.5272	up
984	NM_020530	482	Hs.248156	oncostatin M (OSM), mRNA /cds=(0,758)	4.5272	up
985	NM_003321	483	Hs.12084	Tu translation elongation factor, mitochondrial (TUFM)	4.5631	up
986	BE901218	484	Hs.285122	Homo sapiens, hypothetical protein FLJ21839, clone MGC:2851 IMAGE:2967512, mRNA, complete cds /cds=(444,2618)	4.5631	up
987	AI361733	485	Hs.157811	qz24b02.x1 cDNA, 3' end /clone=IMAGE:2027787 /clone_end=3'	4.5631	up
988	AK026410	486	Hs.236449	hypothetical protein FLJ22757 (FLJ22757), mRNA /cds=(92,2473)	4.6078	up
989	BG254292	487	NA	cDNA clone IMAGE:4477042 5'	4.6078	up
990	NM_001504	488	Hs.198252	G protein-coupled receptor 9 (GPR9), mRNA /cds=(68,1174)	4.6078	up
991	BE964596	489	Hs.184052	PP1201 protein (PP1201), mRNA /cds=(75,1010)	4.6078	up
992	AB011098	490	Hs.59403	serine palmitoyltransferase, long chain base subunit 2 (SPTLC2), mRNA /cds=(188,1876)	4.6346	up
993	BE745645	491	Hs.127951	hypothetical protein FLJ14503 (FLJ14503), mRNA /cds=(19,2217)	4.6346	up
994	AI827950	492	Hs.342617	ha15h10.x1 cDNA, 3' end /clone=IMAGE:2873827 /clone_end=3'	4.6346	up
995	AL521097	493	Hs.13144	HSPC160 protein (HSPC160), mRNA /cds=(53,514)	4.6346	up
996	BE222032	494	Hs.128675	hr61g11.x1 cDNA, 3' end /clone=IMAGE:3133028 /clone_end=3'	4.6346	up
997	AA516406	495	Hs.180201	hypothetical protein FLJ20671 (FLJ20671), mRNA /cds=(72,494)	4.7382	up
998	AJ277832	496	Hs.56247	mRNA for inducible T-cell co-stimulator (ICOS gene) /cds=(67,666)	4.7426	up
999	AV653169	497	Hs.5897	cDNA FLJ13388 fis, clone PLACE1001168 /cds=UNKNOWN	4.7426	up
1000	M36820	498	Hs.75765	GRO2 oncogene (GRO2), mRNA /cds=(74,397)	4.7432	up
1001	NM_015919	499	Hs.145956	zinc finger protein mRNA, complete cds /cds=(1073,3133)	4.7432	up
1002	AI378109	500	Hs.283438	7f19b03.x1 cDNA, 3' end /clone=IMAGE:3295085 /clone_end=3'	4.7432	up
1003	AI436418	501	Hs.369051	ESTs, Weakly similar to VAM5_HUMAN Vesicule-associated membrane protein 5 (VAMP-5) (Myobrevin) (HSPC191)	4.7432	up
1004	NM_022488	502	Hs.26367	PC3-96 protein (PC3-96), mRNA /cds=(119,586)	4.8445	up
845	AI760224	343	Hs.26873	wh62g06.x1 cDNA, 3' end /clone=IMAGE:2385370 /clone_end=3'	0.3142, 0.56	up, down
504	W16552	2	Hs.306117	PKR	0, 0	up
846	AL565736	344	Hs.181165	eukaryotic translation elongation factor 1 alpha 1 (EEF1A1), mRNA /cds=(53,1441)	0.0909, 1.6, 0.090	down
847	NM_004900	345	Hs.226307	phorbolin (similar to apolipoprotein B mRNA editing protein) (DJ742C19.2), mRNA /cds=(79,651)	0.25, 3.09	up

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
848	AI031624	346	Hs.238954	602637935F1 cDNA, 5' end /clone=IMAGE:4765448 /clone_end=5'	0.3142, 0.5638	up, down
849	BF059133	347	Hs.144583	Homo sapiens, clone IMAGE:3462401, mRNA, partial cds /cds=(0,153)	0.669, 0.564	up, down
850	AB036432	348	Hs.184	advanced glycosylation end product-specific receptor (AGER), mRNA /cds=(0,1214)	0.6691, 0.9257	up, down
851	R64054	349	Hs.208603	7f01d11.x1 cDNA, 3' end /clone=IMAGE:3293397 /clone_end=3'	0.6845, 0.926	up, down
852	M81601	350	Hs.153179	fatty acid binding protein 5 (psoriasis-associated) (FABP5), mRNA /cds=(48,455)	1.03, 3.0908	up, down
853	AY004255	351	Hs.238990	Homo sapiens, Similar to cyclin-dependent kinase inhibitor 1B (p27, Kip1)	1.1, 0.0909	down
854	NM_002258	352	Hs.169824	killer cell lectin-like receptor subfamily B, member 1 (KLRB1), mRNA /cds=(60,737)	1.1, 0.1071	down
855	M11124	353	Hs.198253	major histocompatibility complex, class II, DQ alpha 1 (HLA-DQA1), mRNA /cds=(43,810)	1.1, 0.193	down
514	NM_002946	12	Hs.79411	replication protein A2 (32kD) (RPA2)	1.1, 0.4174	down
856	AF073705	354	Hs.247721	clone mcg53-54 immunoglobulin lambda light chain variable region 4a mRNA, partial cds /cds=(0,324)	1.1, 0.9542	up
857	AJ271326	355	Hs.135187	unc93 (C.elegans) homolog B (UNC93B), mRNA /cds=(41,1834)	1.1, 1.437	up
858	NM_138391	356	Hs.17481	mRNA; cDNA DKFZp434G2415 (from clone DKFZp434G2415) /cds=UNKNOWN	1.1, 2.246	up
859	X97324	357	Hs.3416	adipose differentiation-related protein (ADFP), mRNA /cds=(0,1313)	1.1, 2.32	up
507	NM_006187	5	Hs.56009	2'-5'-oligoadenylate synthetase 3 (100 kD) (OAS3), mRNA /cds=(34,3297)	1.1, 2.47	up
860	NM_006289	358	Hs.18420	talin 1 (TLN1), mRNA /cds=(126,7751)	1.1, 3.0908	up
861	NM_002935	359	Hs.73839	ribonuclease, RNase A family, 3 (eosinophil cationic protein) (RNASE3), mRNA /cds=(63,545)	1.1, 4.56	up
862	Y00345	360	Hs.172182	poly(A)-binding protein, cytoplasmic 1 (PABPC1), mRNA /cds=(502,2403)	1.6, 0.0909	down
863	AL567986	361	Hs.77393	farnesyl diphosphate synthase	1.6, 0.0909	down
864	NM_000311	362	Hs.74621	prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome)	1.6, 0.141	down
865	NM_016523	363	Hs.183125	killer cell lectin-like receptor subfamily F, member 1 (KLRF1), mRNA /cds=(64,759)	1.6, 0.213	down

Table 2A: Significance analysis for Microarrays, Lupus/Autoimmune merkers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	SAM FDR	SAM Up/Down
866	AA701193	364	Hs.431104	EST, Weakly similar to HA21_HUMAN HLA class II histocompatibility antigen, DQ(1) alpha chain precursor (DC-4 alpha chain)	1.6, 0.2759	down
510	NM_004510	8	Hs.38125	interferon-induced protein 75, 52kD (IFI75), mRNA /cds=(170,1396)	1.6, 1.12	up
867	AK026594	365	Hs.251653	tubulin, beta, 2 (TUBB2), mRNA /cds=(0,1337)	1.6, 2.32	up
503	NM_000389	1	Hs.179665	CDKN1A cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.6, 3.09	up
868	AW063509	366	Hs.279105	TN1012 cDNA, 3' end /clone_end=3'	1.6, 3.09	up
869	R14692	367	Hs.170222	Na+/H+ exchanger NHE-1 isoform [human, heart, mRNA, 4516 nt] /cds=(577,3024)	1.6, 3.33	up
870	NM_002831	368	Hs.63489	protein tyrosine phosphatase, non-receptor type 6 (PTPN6), mRNA /cds=(144,1931)	1.6, 4.3699	up
871	BE868389	369	Hs.179703	tripartite motif protein 14 (TRIM14), mRNA /cds=(10,1230)	1.6, 4.6	up
509	BC002409	7	Hs.288061	actin, beta (ACTB), mRNA	NA	
518	NM_003033	16	Hs.301698	BAC 180i23 chromosome 8 map 8q24.3 beta-galactoside alpha-2,3-sialyltransferase (SIAT4A) gene	NA	
872	AK090404	370	Hs.98531	53G7, FLJ00290 protein	NA	
873	AK024202	371	Hs.289088	heat shock 90kD protein 1, alpha (HSPCA), mRNA /cds=(60,2258)	NA	
874	AK024240	372	Hs.24115	cDNA FLJ14178 fis, clone NT2RP2003339 /cds=UNKNOWN	NA	
875	AK024756	373	Hs.12293	hypothetical protein FLJ21103 (FLJ21103), mRNA /cds=(88,1143)	NA	
876	AK024969	374	Hs.166254	hypothetical protein DKFZp566I133 (DKFZP566I133), mRNA /cds=(133,1353)	NA	
877	AL136542	375	Hs.322456	hypothetical protein DKFZp761D0211 (DKFZP761D0211), mRNA /cds=(164,1822)	NA	
878	NM_015995	376	Hs.7104	mRNA; cDNA DKFZp761P06121 (from clone DKFZp761P06121) /cds=UNKNOWN	NA, 3.09	up

Table 2B: Real-time PCR gene expression analysis

SEQ ID 50mer	ACC	SEQ ID FL	HS	Gene	PCR Fold	PCR p-value	SEQ ID forward	Forward primer	SEQ ID reverse	Reverse primer
503	NM_000389	1	Hs.179665	CDKN1A cyclin-dependent kinase inhibitor 1A (p21, Cip1)	2.25	0.0000	1005	CTAACGTTGAGCCCCTGGAG	1006	ATGGGGAGCCGAGAGAAAAC
504	W16552	2	Hs.306117	PKR	2.60	0.0000	1007	TCGACATGGTGAGGTAGAGCA	1008	TGTTCTGGCAGCACCTCAAG
505	NM_004031	3	Hs.166120	interferon regulatory factor 7 (IRF7), transc	2.76	0.0001	1009	AGCGTGAGGGTGTGTCTTCC	1010	GGCTGCTCCAGCTCCATAAG
506	NM_006417	4	Hs.82316	interferon-induced, hepatitis C-associated	4.34	0.0001	1011	TGGGAGCTGGACCCTGTAAA	1012	GCAGCCCATAGCATTCGTCT
507	NM_006187	5	Hs.56009	2'-5'-oligoadenylate synthetase 3 (100 kD) (OAS3)	5.40	0.0001	1013	CGCAGTTGGGTACCTTCCAT	1014	TGCTCTGGTTCCCACCATCT
508	NM_001548	6	Hs.20315	interferon-induced protein with tetratricopeptide repeats 1	10.30	0.0019	1015	CTGGAAAGCTTGAGCCTCCTT	1016	CTCAGGGCCCGCTCATAGTA
509	BC002409	7	Hs.288061	actin, beta (ACTB), mRNA	1.29	0.0028	1017	CACAATGTGGCCGAGGACTT	1018	TGGCTTTTAGGATGGCAAGG
510	NM_004510	8	Hs.38125	interferon-induced protein 75, 52kD (IFI75)	1.36	0.0034	1019	CAAAGACGTGCTCGGTTTTCA	1020	TGAATCCTGAGGTGGGGATG
511	NM_000269	9	Hs.183698	ribosomal protein L29 (RPL29)	1.38	0.0057	1021	CATCCATTTCCCCTCCTTCC	1022	CAGATGGTCGGGGATGGTAA
512	NM_138391	10	Hs.17481	Homo sapiens chromosome 1 open reading frame 37 (C1orf37)	1.15	0.0160	1023	TCTTGGAGATTCGAGCAGCA	1024	CTGCGACCAGAGTCAGTGGA
513	BC036402	11	NA	116C9	2.26	0.0258	1025	CCTGATTCGCCAATTTGTCC	1026	CCCAACCCCAAAATCCCTAA
514	NM_002946	12	Hs.79411	replication protein A2 (32kD) (RPA2)	0.88	0.0458	1027	CGTCATGGCAAGTGTGTCAA	1028	TGGCCTCTGCCTGTTTTCAT
515	NM_031157	13	Hs.249495	heterogeneous nuclear ribonucleoprotein A1 (HNRPA1)	0.79	0.0538	1029	TGGTAAATTTCCCCAACAGTGTG	1030	CACCAAGGTTTCCGAAGACAA
516	D23660	14	Hs.334822	Homo sapiens, Similar to ribosomal protein L4	0.73	0.0650	1031	AGCACCACGCAAGAAGATCC	1032	CTGGCGAAGAATGGTGTTCC
517	NM_006276	15	Hs.184167	splicing factor, arginine/serine-rich 7 (35kD) (SFRS7)	0.85	0.3054	1033	TTGCGCAGATACCTAGGCTTG	1034	TCAGCCAGTCAAAATTCCAAAA
518	NM_003033	16	Hs.301698	beta-galactoside alpha-2,3-sialyltransferase (SIAT4A) gene	0.88	0.3680	1035	ACCCATCTACCGGCATCCTC	1036	GTGCCAGTTCCCTTTGCTGT
519	BE550944	17	Hs.61426	602329933F1 cDNA	0.95	0.5085	1037	CAAAACCTCGCTTACTGTCATGTG	1038	TGGGAAAGGACATCAGTCTTCA

Table 2C: Multiple Additive Regression Trees analysis of Microarray Data

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	MART Importance	MART error	Imp/error
515	NM_031157	13	Hs.249495	heterogeneous nuclear ribonucleoprotein A1 (HNRPA1), transcript variant 2, mRNA /cds=(104,1222)	68.5	0.202	339.108911
516	D23660	14	Hs.334822	Homo sapiens, Similar to ribosomal protein L4, clone MGC:2966 IMAGE:3139805, mRNA, complete cds /cds=(1616,2617)	68.71	0.202	340.148515
519	BE550944	17	Hs.61426	602329933F1 cDNA	100, 66.33	0.202	347.0, 328.22
537	NM_000734	35	Hs.97087	CD3Z antigen, zeta polypeptide (TiT3 complex) (CD3Z), mRNA /cds=(178,669)	67.49	0.202	334.108911
538	NM_003756	36	Hs.58189	eukaryotic translation initiation factor 3, subunit 3 (gamma, 40kD) (EIF3S3), mRNA /cds=(5,1063)		0.092	996.521739
624	NM_006791	122	Hs.6353	MORF-related gene 15 (MRG15), mRNA /cds=(131,1102)	65.53	0.202	324.405941
517	NM_006276	15	Hs.184167	splicing factor, arginine/serine-rich 7 (35kD) (SFRS7) mRNA /cds=(105,490)	100	0.092	1086.95652
754	AL133642	252	Hs.241471	mRNA; cDNA DKFZp586G1721 (from clone DKFZp586G1721); partial cds /cds=(0,669)	42.88	0.288	148.888889
797	AL050371	295	Hs.8128	phosphatidylserine decarboxylase (PISD), mRNA /cds=(223,1350)	70.07	0.288	243.298611
927	NM_003264	425	Hs.63668	toll-like receptor 2 (TLR2), mRNA /cds=(129,2483)	49.97	0.288	173.506944
845	AI760224	343	Hs.26873	wh62g06.x1 cDNA, 3' end /clone=IMAGE:2385370	49.83	0.288	173.020833
862	Y00345	360	Hs.172182	poly(A)-binding protein, cytoplasmic 1 (PABPC1), mRNA /cds=(502,2403)	31.53	0.202	156.089109
511	NM_000269	9	Hs.183698	ribosomal protein L29 (RPL29), mRNA /cds=(29,508)	99.34	0.092	1079.78261
882	NM_003128	380	Hs.324648	cDNA FLJ13700 fis, clone PLACE2000216, highly similar to SPECTRIN BETA CHAIN, BRAIN /cds=UNKNOWN	100	0.202	495.049505
883	AL109669	381	Hs.172803	mRNA full length insert cDNA clone EUROIMAGE 31839 /cds=UNKNOWN	55.24	0.202	273.465347
884	AI307808	382	Hs.238797	602081661F1 cDNA, 5' end /clone=IMAGE:4245999	33.2	0.202	164.356436
885	AF261087	383	Hs.174131	ribosomal protein L6 (RPL6), mRNA /cds=(26,892)	0.68	0.202	3.36633663
886	NM_002546	384	Hs.81791	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) (TNFRSF11B), mRNA /cds=(94,1299)	48.54	0.202	240.29703
887	NM_012237	385	Hs.44017	sirtuin (silent mating type information regulation 2, S.cerevisiae, homolog) 2 (SIRT2), transcript variant 1, mRNA /cds=(200,1369)	68.21	0.202	337.673267
888	X68060	386	Hs.75248	topoisomerase (DNA) II beta (180kD) (TOP2B), mRNA /cds=(0,4865)	48.14	0.288	167.152778

Table 2C: Multiple Additive Regression Trees analysis of Microarray Data

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	MART Importance	MART error	Imp/error
889	AI660405	387	Hs.111941	qd92a04.x1 cDNA, 3' end /clone=IMAGE:1736910	51.11	0.288	177.465278
890	AI798114	388	Hs.210307	wh81c01.x1 cDNA, 3' end /clone=IMAGE:2387136	0.96	0.202	4.75247525
891	AW075948	389	Hs.265634	xa82b03.x1 cDNA, 3' end /clone=IMAGE:2573261	50.06	0.202	247.821782
892	AW294681	390	Hs.255336	UI-H-BW0-ail-g-10-0-UI.s1 cDNA, 3' end /clone=IMAGE:2729683 /clone_end=3'	50.28	0.384	130.9375
893	R40823	391	Hs.108082	602068988F1 cDNA, 5' end /clone=IMAGE:4067972	32.33	0.202	160.049505
894	AA806222	392	Hs.111554	ADP-ribosylation factor-like 7 (ARL7), mRNA /cds=(14,592)	44.47	0.288	154.409722
895	AI380390	393	Hs.158976	UI-H-BI2-ahi-a-03-0-UI.s1 cDNA, 3' end /clone=IMAGE:2726692 /clone_end=3'	54.86	0.202	271.584158
896	BF435621	394	Hs.293476	hypothetical protein FKSG44 (FKSG44), mRNA /cds=(126,1520)	100	0.285	350.877193
897	AK025781	395	Hs.5076	cDNA: FLJ22128 fis, clone HEP19543 /cds=UNKNOWN	51.37	0.288	178.368056
898	X06323	396	Hs.79086	mitochondrial ribosomal protein L3 (MRPL3), mRNA /cds=(76,1122)	47.57	0.288	165.173611
899	X72841	397	Hs.31314	retinoblastoma-binding protein 7 (RBBP7), mRNA /cds=(287,1564)	50.46	0.288	175.208333

Table 2D: Hierarchical Clustering of Lupus/Autoimmunity Markers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	Hierarchical Cluster OID
900	NM_001015	398	Hs.182740	ribosomal protein S11 (RPS11), mRNA /cds=(15,4	180
901	J02931	399	Hs.62192	placental tissue factor (two forms) mRNA, complete cd	180
914	NM_001778	412	Hs.901	CD48 antigen (B-cell membrane protein) (CD48), mRNA /cds=(36,767)	180
862	Y00345	360	Hs.172182	poly(A)-binding protein, cytoplasmic 1 (PABPC1), mRNA /cds=(502,2403)	2177
524	NM_001731	22	Hs.77054	B-cell translocation gene 1, anti-proliferative (BTG1), mRNA /cds=(308,823)	2177
528	U67093	26	Hs.194382	ataxia telangiectasia (ATM) gene, complete cds /cds=(795,9965)	2177
529	AJ400717	27	Hs.279860	tumor protein, translationally-controlled 1 (TPT1), mRNA /cds=(94,612)	2177
920	NM_014065	418	Hs.279040	HT001 protein (HT001), mRNA /cds=(241,1203)	3780
563	U61267	61	Hs.30035	putative splice factor transformer2-beta mRN	5067
680	BF897042	178	Hs.120219	FLJ32028 hypothetical protein FLJ32028	5067
506	NM_006417	4	Hs.82316	interferon-induced, hepatitis C-associated microtubular aggregate protein (44kD) (MTAP44), mRNA /cds=(0,1334)	5067
504	W16552	2	Hs.306117	PKR	5067
507	NM_006187	5	Hs.56009	2'-5'-oligoadenylate synthetase 3 (100 kD) (OAS3), mRNA /cds=(34,3297)	5067
715	NM_001295	213	Hs.301921	chemokine (C-C motif) receptor 1 (CCR1), mRNA /cds=(62,1129)	5067
739	NM_022873	237	Hs.265827	interferon, alpha-inducible protein (clone IFI-6-16) (G1P3), transcript variant 3, mRNA /cds=(107,523)	5067
505	NM_004031	3	Hs.166120	interferon regulatory factor 7 (IRF7), transc	5067
508	NM_001548	6	Hs.20315	interferon-induced protein with tetratricopeptide repeats 1 (IFIT1)	5067
904	AF307339	402	Hs.47783	B aggressive lymphoma gene (BAL), mRNA /cds=(228,2792)	5067
906	AK027260	404	Hs.152925	mRNA for KIAA1268 protein, partial cds /cds=(0,3071)	5067
907	AL360190	405	Hs.318501	stimulated trans-acting factor (50 kDa) (STAF50), mRNA /cds=(122,1450)	5067
917	NM_004031	415	Hs.166120	interferon regulatory factor 7 (IRF7), transcript variant d, mRNA /cds=(335,1885)	5067
921	NM_017523	419	Hs.139262	XIAP associated factor-1 (HSXIAPAF1), mRNA /cds=(0,953)	5067
922	NM_021105	420	Hs.198282	phospholipid scramblase 1 (PLSCR1), mRNA /cds=(256,1212)	5067

Table 2D: Hierarchical Clustering of Lupus/Autoimmunity Markers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	Hierarchical Cluster OID
923	XM_005543	421	Hs.234642	aquaporin 3 (AQP3), mRNA /cds=(64,942)	5067
642	W00466	140	Hs.44189	yz99f01.s1 cDNA, 3' end /clone=IMAGE:291193 /clone_end=3'	5083
679	AW137104	177	Hs.8121	Notch (Drosophila) homolog 2 (NOTCH2), mRNA /cds=(12,7427)	5083
848	AI031624	346	Hs.238954	602637935F1 cDNA, 5' end /clone=IMAGE:4765448 /clone_end=5'	5083
677	NM_003367	175	Hs.93649	upstream transcription factor 2, c-fos intera	6382
503	NM_000389	1	Hs.179665	CDKN1A cyclin-dependent kinase inhibitor 1A (p21, Cip1)	6382
515	NM_031157	13	Hs.249495	heterogeneous nuclear ribonucleoprotein A1 (HNRPA1), transcript variant 2, mRNA /cds=(104,1222)	6444
516	D23660	14	Hs.334822	Homo sapiens, Similar to ribosomal protein L4, clone MGC:2966 IMAGE:3139805, mRNA, complete cds /cds=(1616,2617)	6444
520	L13385	18	Hs.77318	Miller-Dieker lissencephaly protein (LIS1)	6444
527	XM_018498	25	Hs.180946	ribosomal protein L5 pseudogene mRNA, complete cds /cds=UNKNOWN	6444
512	NM_138391	10	Hs.17481	Homo sapiens chromosome 1 open reading frame 37 (C1orf37), mRNA	6956
902	D49950	400	Hs.83077	for interferon-gamma inducing activated macrophages	6956
908	AV689330	406	Hs.189402	Similar to RIKEN cDNA 2210009G21 gene, clone IMAGE:4807023	6956
909	BC002796	407	Hs.46446	lymphoblastic leukemia derived sequence 1 (LYL1), mRNA /cds=(0,803)	6956
910	BE899595	408	NA	cDNA clone IMAGE:3952215 5'	6956
912	NM_001111	410	Hs.7957	adenosine deaminase, RNA-specific (ADAR), transcript variant ADAR-a, mRNA /cds=(187,3867)	6956
915	NM_002463	413	Hs.926	myxovirus (influenza) resistance 2, homolog of murine (MX2), mRNA /cds=(104,2251)	6956
918	NM_006865	416	Hs.113277	leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 3 (LILRA3), mRNA /cds=(62,1381)	6956
919	NM_013352	417	Hs.58636	squamous cell carcinoma antigen recognized by T cell (SART-2), mRNA /cds=(149,3025)	6956
924	NM_009587	422	Hs.81337	lectin, galactoside-binding, soluble, 9 (galectin 9) (LGALS9), transcript variant long, mRNA /cds=(56,1123)	6956

Table 2D: Hierarchical Clustering of Lupus/Autoimmunity Markers

SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene	Hierarchical Cluster OID
807	NM_000395	305	Hs.285401	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage) (CSF2RB), mRNA /cds=(28,2721)	7330
950	NM_002462	448	Hs.76391	myxovirus (influenza) resistance 1, homolog of murine (interferon-inducible protein p78) (MX1), mRNA /cds=(345,2333)	7330
905	AK024597	403	Hs.10362	cDNA: FLJ20944 fis, clone ADSE01780 /cds=UNKNOWN	7330
913	NM_001549	411	Hs.181874	interferon-induced protein with tetratricopeptide repeats 4 (IFIT4), mRNA /cds=(61,1533)	7330
916	NM_002759	414	Hs.274382	protein kinase, interferon-inducible double stranded RNA dependent (PRKR), mRNA /cds=(435,2090)	7330
911	K02766	409	Hs.1290	complement component 9 (C9), mRNA /cds=(4,1683)	7379
813	M26683	311	Hs.303649	interferon gamma treatment inducible mRNA Monocytes	7238, 6956
903	NM_001772	401	Hs.83731	CD33 antigen (gp67) (CD33), mRNA.	7238, 6956

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
6382	503	NM_000389	1	Hs.179665	CDKN1A cyclin-dependent kinase inhibitor 1A (p21, Cip1)
5067	504	W16552	2	Hs.306117	PKR
41	505	NM_004031	3	Hs.166120	interferon regulatory factor 7 (IRF7), transc
2922	506	NM_006417	4	Hs.82316	interferon-induced, hepatitis C-associated microtubular aggregate protein
7238	507	NM_006187	5	Hs.56009	2'-5'-oligoadenylate synthetase 3 (100 kD) (OAS3), mRNA /cds=(34,3297)
2139	508	NM_001548	6	Hs.20315	interferon-induced protein with tetratricopeptide repeats 1 (IFIT1)
1436	509	BC002409	7	Hs.288061	actin, beta (ACTB), mRNA
2648	510	NM_004510	8	Hs.38125	interferon-induced protein 75, 52kD (IFI75), mRNA /cds=(170,1396)
7576	511	NM_000269	9	Hs.183698	ribosomal protein L29 (RPL29), mRNA /cds=(29,508)
6956	512	NM_138391	10	Hs.17481	Homo sapiens chromosome 1 open reading frame 37 (C1orf37), mRNA
6957	513	BC036402	11	NA	116C9
2412	514	NM_002946	12	Hs.79411	replication protein A2 (32kD) (RPA2)
6444	515	NM_031157	13	Hs.249495	heterogeneous nuclear ribonucleoprotein A1 (HNRPA1), transcript variant 2, mRNA /cds=(104,1222)
7991	516	D23660	14	Hs.334822	Homo sapiens, Similar to ribosomal protein L4
4143	517	NM_006276	15	Hs.184167	splicing factor, arginine/serine-rich 7 (35kD) (SFRS7) mRNA /cds=(105,490)
4637	518	NM_003033	16	Hs.301698	BAC 180i23 chromosome 8 map 8q24.3 beta-galactoside alpha-2,3-sialyltransferase (SIAT4A) gene
6335	519	BE550944	17	Hs.61426	602329933F1 cDNA
219	520	L13385	18	Hs.77318	Miller-Dieker lissencephaly protein (LIS1)
827	521	AF315591	19	Hs.6151	pumilio (Drosophila) homolog 2 (PUM2)
1064	522	AK025620	20	Hs.5985	cDNA: FLJ21967 fis, clone HEP05652, highly similar to AF131831 clone 25186 mRNA sequence
1125	523	AK026747	21	Hs.12969	cDNA: FLJ23094 fis, clone LNG07379
2177	524	NM_001731	22	Hs.77054	B-cell translocation gene 1, anti-proliferative (BTG1), mRNA /cds=(308,823)
2621	525	NM_004281	23	Hs.15259	BCL2-associated athanogene 3 (BAG3), mRNA /cds=(306,2033)
4114	526	XM_008738	24	Hs.79241	B-cell CLL/lymphoma 2 (BCL2), nuclear gene encoding mitochondrial protein, transcript variant alpha, mRNA /cds=(31,750)
4141	527	XM_018498	25	Hs.180946	ribosomal protein L5 pseudogene mRNA, complete cds /cds=UNKNOWN
4142	528	U67093	26	Hs.194382	ataxia telangiectasia (ATM) gene, complete cds /cds=(795,9965)
7959	529	AJ400717	27	Hs.279860	tumor protein, translationally-controlled 1 (TPT1), mRNA /cds=(94,612)
2459	530	NM_003133	28	Hs.75975	signal recognition particle 9kD (SRP9), mRNA /cds=(106,366)
2620	531	NM_004261	29	Hs.90606	15 kDa selenoprotein (SEP15), mRNA /cds=(4,492)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
2278	532	NM_002300	30	Hs.234489	Homo sapiens, lactate dehydrogenase B, clone MGC:3600 IMAGE:3028947, mRNA, complete cds
4653	533	NM_003853	31	Hs.158315	interleukin 18 receptor accessory protein (IL18RAP), mRNA /cds=(483,2282)
8056	534	X53777	32	Hs.82202	ribosomal protein L17 (RPL17), mRNA /cds=(286,840)
1864	535	N27575	33	Hs.75613	CD36 antigen (collagen type I receptor, thrombospondin receptor) (CD36), mRNA /cds=(132,1550)
2963	536	NM_006800	34	Hs.88764	male-specific lethal-3 (Drosophila)-like 1 (MSL3L1), mRNA /cds=(105,1670)
1991	537	NM_000734	35	Hs.97087	CD3Z antigen, zeta polypeptide (TiT3 complex) (CD3Z), mRNA /cds=(178,669)
2547	538	NM_003756	36	Hs.58189	eukaryotic translation initiation factor 3, subunit 3 (gamma, 40kD) (EIF3S3), mRNA /cds=(5,1063)
275	539	NM_021950	37	Hs.89751	CD20 antigen
921	540	AK021632	38	Hs.11571	cDNA FLJ11570 fis, clone HEMBA1003309 /cds=UNKNOWN
1061	541	AK025583	39	Hs.82845	cDNA: FLJ21930 fis, clone HEP04301, highly similar to HSU90916 clone 23815 mRNA sequence
1987	542	NM_000661	40	Hs.157850	Homo sapiens, clone MGC:15545 IMAGE:3050745, mRNA, complete cds /cds=(1045,1623)
4552	543	NM_001057	41	Hs.161305	tachykinin receptor 2 (TACR2), mRNA /cds=(0,1196)
4903	544	X60656	42	Hs.275959	eukaryotic translation elongation factor 1 beta 2 (EEF1B2), mRNA /cds=(235,912)
2687	545	NM_004779	43	Hs.26703	CNOT8 CCR4-NOT transcription complex, subunit 8
3763	546	X58529	44	Hs.302063	rearranged immunoglobulin mRNA for mu heavy chain enhancer and constant region /cds=UNKNOWN
3262	547	NM_016091	45	Hs.119503	HSPC025 (HSPC025), mRNA /cds=(33,1727)
218	548	NM_001006	46	Hs.77039	ribosomal protein S3A (RPS3A), mRNA /cds=(36,8
2150	549	NM_001568	47	Hs.106673	eukaryotic translation initiation factor 3, subunit 6 (48kD) (EIF3S6), mRNA /cds=(22,1359)
221	550	BC001854	48	Hs.77502	, methionine adenosyltransferase II, alpha, c
2032	551	NM_000983	49	Hs.326249	ribosomal protein L22 (RPL22), mRNA /cds=(51,437)
2046	552	NM_001006	50	Hs.155101	mRNA for KIAA1578 protein, partial cds /cds=(0,3608)
2113	553	NM_001403	51	Hs.274466	eukaryotic translation elongation factor 1 alpha 1-like 14 (EEF1A1L14), mRNA /cds=(620,1816)
2374	554	NM_002796	52	Hs.89545	proteasome (prosome, macropain) subunit, beta type, 4 (PSMB4), mRNA /cds=(23,817)
3290	555	NM_016304	53	Hs.284162	60S ribosomal protein L30 isolog (LOC51187), mRNA /cds=(143,634)
3353	556	NM_017918	54	Hs.234149	hypothetical protein FLJ20647 (FLJ20647), mRNA /cds=(90,836)
4192	557	AA788623	55	Hs.332583	yc77a06.s1 cDNA, 3' end /clone=IMAGE:21844 /clone_end=3'

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
7248	558	NM_001961	56	Hs.75309	eukaryotic translation elongation factor 2 (EEF2), mRNA /cds=(0,2576)
7631	559	AK026309	57	Hs.12436	cDNA: FLJ22656 fis, clone HSI07655 /cds=UNKNOWN
1112	560	AK026528	58	Hs.334807	Homo sapiens, ribosomal protein L30, clone MGC:2797, mRNA, complete cds /cds=(29,376)
1450	561	BC002971	59	Hs.1600	Homo sapiens, clone IMAGE:3543711, mRNA, partial cds /cds=(0,1620)
3572	562	U01923	60	Hs.278857	heterogeneous nuclear ribonucleoprotein H2 (H') (HNRPH2), mRNA /cds=(78,1427)
140	563	U61267	61	Hs.30035	putative splice factor transformer2-beta mRN
220	564	X14356	62	Hs.77424	high affinity Fc receptor (FcRI) /cds=(36,116
809	565	AF267856	63	Hs.8084	HT033 mRNA, complete cds /cds=(203,931)
1048	566	AK025306	64	Hs.2083	cDNA: FLJ21653 fis, clone COL08586, highly similar to HUMKINCDC protein kinase mRNA /cds=UNKNOWN
1262	567	AL162068	65	Hs.302649	HSP22-like protein interacting protein (LOC64165), mRNA /cds=(0,155)
2685	568	NM_004768	66	Hs.11482	splicing factor, arginine/serine-rich 11 (SFRS11), mRNA /cds=(83,1537)
2793	569	NM_005594	67	Hs.158164	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) (TAP1), mRNA /cds=(30,2456)
5210	570	AI440234	68	Hs.9614	Nucleophosmin (probe bad, mutations, wrong clone used) (nucleolar phosphoprotein B23, numatrin)
5732	571	AW194379	69	Hs.203755	xm08h07.x1 cDNA, 3' end /clone=IMAGE:2683645 /clone_end=3'
2829	572	NM_005826	70	Hs.15265	heterogeneous nuclear ribonucleoprotein R (HNRPR), mRNA /cds=(90,1991)
5318	573	AI568695	71	Hs.75969	proline-rich protein with nuclear targeting signal (B4-2), mRNA /cds=(113,1096)
7965	574	AL110225	72	Hs.89434	drebrin 1 (DBN1), mRNA /cds=(97,2046)
1198	575	AL110151	73	Hs.128797	mRNA; cDNA DKFZp586D0824 (from clone DKFZp586D0824); partial cds /cds=(0,1080)
2933	576	NM_006495	74	Hs.5509	ecotropic viral integration site 2B (EVI2B), mRNA /cds=(0,1346)
1846	577	M74002	75	Hs.11482	splicing factor, arginine/serine-rich 11 (SFRS11), mRNA /cds=(83,1537)
917	578	AK002173	76	Hs.5518	cDNA FLJ11311 fis, clone PLACE1010102 /cds=UNKNOWN
1037	579	AK024976	77	Hs.323378	coated vesicle membrane protein (RNP24), mRNA /cds=(27,632)
1415	580	BC000967	78	Hs.195870	chronic myelogenous leukemia tumor antigen 66 mRNA, complete cds, alternatively spliced /cds=(232,1983)
3291	581	NM_016312	79	Hs.334811	Npw38-binding protein NpwBP (LOC51729), mRNA /cds=(143,2068)
3759	582	X57347	80	Hs.74405	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide (YWHAQ), mRNA /cds=(100,837)
4045	583	BG424974	81	Hs.292457	Homo sapiens, clone MGC:16362 IMAGE:3927795, mRNA, complete cds /cds=(498,635)
4155	584	U89387	82	Hs.194638	polymerase (RNA) II (DNA directed) polypeptide D (POLR2D), mRNA /cds=(30,458)
153	585	AB034205	83	Hs.278670	Acid-inducible phosphoprotein

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
4111	586	XM_008062	84	Hs.17279	tyrosylprotein sulfotransferase 1 (TPST1), mRNA /cds=(81,1193)
3263	587	NM_016099	85	Hs.7953	HSPC041 protein (LOC51125), mRNA /cds=(141,455)
3510	588	NM_022898	86	Hs.57987	B-cell lymphoma/leukaemia 11B (BCL11B), mRNA /cds=(267,2738)
2956	589	NM_006759	87	Hs.77837	UDP-glucose pyrophosphorylase 2 (UGP2), mRNA /cds=(84,1610)
694	590	AF079566	88	Hs.4311	SUMO-1 activating enzyme subunit 2 (UBA2), mRNA /cds=(25,1947)
2055	591	NM_001024	89	Hs.182979	cDNA: FLJ22838 fis, clone KAIA4494, highly similar to HUML12A ribosomal protein L12 mRNA
3336	592	NM_017761	90	Hs.7862	hypothetical protein FLJ20312 (FLJ20312), mRNA /cds=(133,552)
3595	593	U15085	91	Hs.1162	major histocompatibility complex, class II, DM beta (HLA-DMB), mRNA /cds=(233,1024)
6004	594	AW572538	92	Hs.42915	ARP2 (actin-related protein 2, yeast) homolog (ACTR2), mRNA /cds=(74,1258)
1060	595	AK025557	93	Hs.110771	cDNA: FLJ21904 fis, clone HEP03585 /cds=UNKNOWN
4654	596	NM_003854	94	Hs.102865	interleukin 1 receptor-like 2 (IL1RL2), mRNA /cds=(134,1822)
721	597	AF116679	95	Hs.288036	tRNA isopentenylpyrophosphate transferase (IPT), mRNA /cds=(60,1040)
743	598	AF148537	96	Hs.65450	reticulon 4a mRNA, complete cds /cds=(141,3719)
3348	599	NM_017892	97	Hs.107213	hypothetical protein FLJ20585 (FLJ20585), mRNA /cds=(99,746)
2020	600	NM_000967	98	Hs.119598	ribosomal protein L3 (RPL3), mRNA /cds=(6,1217)
6847	601	NM_000971	99	Hs.153	ribosomal protein L7 (RPL7), mRNA /cds=(10,756)
626	602	AF012872	100	Hs.334874	phosphatidylinositol 4-kinase 230 (pi4K230) mRNA, complete cds /cds=(0,6134)
1469	603	BC004900	101	Hs.151242	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1 (SERPING1), mRNA /cds=(60,1562)
2277	604	NM_002298	102	Hs.76506	lymphocyte cytosolic protein 1 (L-plastin) (LCP1), mRNA /cds=(173,2056)
3765	605	X59405	103	Hs.83532	H.sapiens, gene for Membrane cofactor protein /cds=UNKNOWN
4444	606	AL049935	104	Hs.301763	mRNA; cDNA DKFZp564O1116 (from clone DKFZp564O1116) /cds=UNKNOWN
3431	607	NM_017860	105	Hs.79457	hypothetical protein FLJ20519 (FLJ20519), mRNA /cds=(74,604)
1686	608	J04142	106	Hs.1799	CD1D antigen, d polypeptide (CD1D), mRNA /cds=(164,1171)
3267	609	NM_016127	107	Hs.279921	HSPC035 protein (LOC51669), mRNA /cds=(16,1035)
970	610	AK023379	108	Hs.155160	Homo sapiens, Similar to splicing factor, arginine/serine-rich 2 (SC-35)
1710	611	L11284	109	Hs.3446	mitogen-activated protein kinase kinase 1 (MAP2K1), mRNA /cds=(72,1253)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
2358	612	NM_002710	110	Hs.79081	protein phosphatase 1, catalytic subunit, gamma isoform (PPP1CC), mRNA /cds=(154,1125)
2635	613	NM_004380	111	Hs.23598	CREB binding protein (Rubinstein-Taybi syndrome) (CREBBP), mRNA /cds=(198,7526)
8112	614	AW028193	112	Hs.135872	wv61h08.x1 cDNA, 3' end /clone=IMAGE:2534079 /clone_end=3'
2124	615	NM_001436	113	Hs.99853	fibrillarin (FBL), mRNA /cds=(59,1024)
453	616	AB007916	114	Hs.214646	mRNA for KIAA0447 protein, partial cds /cds=(233,1633)
1250	617	AL137681	115	Hs.173912	eukaryotic translation initiation factor 4A, isoform 2 (EIF4A2), mRNA /cds=(15,1238)
1451	618	BC003090	116	Hs.75193	COP9 homolog (COP9), mRNA /cds=(49,678)
3596	619	U15173	117	Hs.155596	BCL2/adenovirus E1B 19kD-interacting protein 2 (BNIP2), mRNA /cds=(211,1155)
3127	620	NM_014210	118	Hs.70499	ecotropic viral integration site 2A (EVI2A), mRNA /cds=(219,917)
2049	621	NM_001011	119	Hs.301547	ribosomal protein S7 (RPS7), mRNA /cds=(81,665)
3585	622	U07802	120	Hs.78909	Tis11d gene, complete cds /cds=(291,1739)
5466	623	AI817153	121	Hs.86693	EST380760 cDNA
2962	624	NM_006791	122	Hs.6353	MORF-related gene 15 (MRG15), mRNA /cds=(131,1102)
4678	625	NM_004500	123	Hs.182447	heterogeneous nuclear ribonucleoprotein C (C1/C2) (HNRPC), transcript variant 1, mRNA /cds=(191,1102)
1768	626	M16660	124	Hs.318720	Homo sapiens, clone MGC:12387 IMAGE:3933019, mRNA, complete cds /cds=(63,863)
2044	627	NM_001000	125	Hs.300141	cDNA FLJ14163 fis, clone NT2RP1000409 /cds=UNKNOWN
4486	628	BC008737	126	Hs.164280	Homo sapiens, Similar to solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5, clone MGC:3042 IMAGE:3342722, mRNA, complete cds /cds=(88,984)
6028	629	BE222392	127	Hs.79914	lumican (LUM), mRNA /cds=(84,1100)
7958	630	BC010112	128	Hs.79037	Homo sapiens, heat shock 60kD protein 1 (chaperonin), clone MGC:19755 IMAGE:3630225, mRNA, complete cds /cds=(1705,3396)
1062	631	AK025586	129	Hs.27268	cDNA: FLJ21933 fis, clone HEP04337 /cds=UNKNOWN
3220	632	NM_015057	130	Hs.151411	KIAA0916 protein (KIAA0916), mRNA /cds=(146,14071)
3590	633	U10550	131	Hs.79022	GTP-binding protein overexpressed in skeletal muscle (GEM), mRNA /cds=(213,1103)
2035	634	NM_000986	132	Hs.184582	DPP7 alveolar r
2039	635	NM_000993	133	Hs.184014	ribosomal protein L31 (RPL31), mRNA /cds=(7,384)
2170	636	NM_001688	134	Hs.81634	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1 (ATP5F1), mRNA
4311	637	AI356505	135	Hs.228874	qz22b04.x1 cDNA, 3' end /clone=IMAGE:2027599 /clone_end=3'

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
7945	638	AF119850	136	Hs.2186	Homo sapiens, eukaryotic translation elongation factor 1 gamma, clone MGC:4501 IMAGE:2964623, mRNA, complete cds /cds=(2278,3231)
739	639	AF132197	137	Hs.301824	hypothetical protein PRO1331 (PRO1331), mRNA /cds=(422,616)
2986	640	NM_006925	138	Hs.166975	splicing factor, arginine/serine-rich 5 (SFRS5), mRNA /cds=(218,541)
4589	641	NM_002001	139	Hs.897	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide (FCER1A), mRNA /cds=(106,879)
5066	642	W00466	140	Hs.44189	yz99f01.s1 cDNA, 3' end /clone=IMAGE:291193 /clone_end=3'
1588	643	D17042	141	Hs.50651	Janus kinase 1 (a protein tyrosine kinase) (JAK1), mRNA /cds=(75,3503)
2505	644	NM_003380	142	Hs.297753	vimentin (VIM), mRNA /cds=(122,1522)
3322	645	NM_016824	143	Hs.324470	adducin 3 (gamma) (ADD3), transcript variant 1, mRNA /cds=(31,2151)
5332	646	AI581383	144	Hs.327922	to71c02.x1 cDNA, 3' end /clone=IMAGE:2183714 /clone_end=3'
4165	647	BC005913	145	Hs.1074	surfactant, pulmonary-associated protein C (SFTPC), mRNA /cds=(27,620)
4691	648	NM_004811	146	Hs.49587	leupaxin (LPXN), mRNA /cds=(93,1253)
1265	649	AL357536	147	Hs.3576	Homo sapiens, Similar to RIKEN cDNA 5730494N06 gene, clone MGC:13348 IMAGE:4132400, mRNA, complete cds /cds=(132,494)
3501	650	NM_022570	148	Hs.161786	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 12 (CLECSF12), mRNA /cds=(71,676)
2636	651	NM_004396	149	Hs.76053	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68kD) (DDX5), mRNA /cds=(170,2014)
1101	652	AK026372	150	Hs.143631	cDNA: FLJ22719 fis, clone HSI14307 /cds=UNKNOWN
4131	653	XM_012059	151	Hs.154938	hypothetical protein MDS025 (MDS025), mRNA /cds=(5,769)
4130	654	XM_011914	152	Hs.180450	ribosomal protein S24 (RPS24), transcript variant 1, mRNA /cds=(37,429)
8031	655	NM_020414	153	Hs.286233	sperm autoantigenic protein 17 (SPA17), mRNA /cds=(1210,1665)
3560	656	S73591	154	Hs.179526	upregulated by 1,25-dihydroxyvitamin D-3 (VDUP1), mRNA /cds=(221,1396)
1675	657	J00194	155	Hs.76807	major histocompatibility complex, class II, DR alpha (HLA-DRA), mRNA /cds=(26,790)
924	658	AK021715	156	Hs.271541	cDNA FLJ11653 fis, clone HEMBA1004538 /cds=UNKNOWN
1144	659	AK027187	157	Hs.289071	cDNA: FLJ22245 fis, clone HRC02612 /cds=UNKNOWN
1212	660	AL117595	158	Hs.4055	mRNA; cDNA DKFZp564C2063 (from clone DKFZp564C2063) /cds=UNKNOWN
2383	661	NM_002823	159	Hs.250655	prothymosin, alpha (gene sequence 28) (PTMA), mRNA /cds=(155,487)
2627	662	NM_004327	160	Hs.234799	breakpoint cluster region (BCR), transcript variant 1, mRNA /cds=(488,4303)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
3343	663	NM_017830	161	Hs.132071	ovarian carcinoma immunoreactive antigen (OCIA), mRNA /cds=(167,904)
3717	664	X06557	162	Hs.2014	mRNA for T-cell receptor delta /cds=UNKNOWN
4244	665	AI146787	163	Hs.156601	qb83f02.x1 cDNA, 3' end /clone=IMAGE:1706715 /clone_end=3'
5323	666	AI568771	164	Hs.327876	th15h04.x1 cDNA, 3' end /clone=IMAGE:2118391 /clone_end=3'
5733	667	AW195119	165	Hs.253151	xn66b07.x1 cDNA, 3' end /clone=IMAGE:2699413 /clone_end=3'
3292	668	NM_016316	166	Hs.110347	REV1 (yeast homolog)- like (REV1L), mRNA /cds=(212,3967)
1324	669	AV724531	167	Hs.76728	602570065F1 cDNA, 5' end /clone=IMAGE:4694321 /clone_end=5'
915	670	AK002059	168	Hs.92918	hypothetical protein (BM-009), mRNA /cds=(385,1047)
2132	671	NM_001503	169	Hs.272529	glycosylphosphatidylinositol specific phospholipase D1 (GPLD1), mRNA /cds=(32,2557)
4221	672	AA251806	170	Hs.177712	zs09c03.s1 cDNA, 3' end /clone=IMAGE:684676 /clone_end=3'
2626	673	NM_004315	171	Hs.75811	N-acylsphingosine amidohydrolase (acid ceramidase) (ASAH), mRNA /cds=(17,1204)
2633	674	NM_004371	172	Hs.75887	coatomer protein complex, subunit alpha (COPA), mRNA /cds=(466,4140)
664	675	AF054284	173	Hs.334826	splicing factor 3b, subunit 1, 155kD (SF3B1), mRNA /cds=(0,3914)
6046	676	BE613237	174	Hs.146381	RNA binding motif protein, X chromosome (RBMX), mRNA /cds=(11,1186)
278	677	NM_003367	175	Hs.93649	upstream transcription factor 2, c-fos intera
469	678	AB014522	176	Hs.11238	mRNA for KIAA0622 protein, partial cds /cds=(0,3869)
5713	679	AW137104	177	Hs.8121	Notch (Drosophila) homolog 2 (NOTCH2), mRNA /cds=(12,7427)
7443	680	BF897042	178	Hs.120219	FLJ32028 hypothetical protein FLJ32028
1447	681	BC002900	179	Hs.181309	proteasome (prosome, macropain) subunit, alpha type, 2 (PSMA2), mRNA /cds=(0,704)
6304	682	AL578975	180	Hs.5057	AL578975 cDNA /clone=CS0DK012YN01-(3-prime)
2037	683	NM_000988	181	Hs.111611	ribosomal protein L27 (RPL27), mRNA /cds=(17,427)
2550	684	NM_003769	182	Hs.77608	splicing factor, arginine/serine-rich 9 (SFRS9), mRNA /cds=(52,717)
3679	685	U94855	183	Hs.7811	eukaryotic translation initiation factor 3, subunit 5 (epsilon, 47kD) (EIF3S5), mRNA /cds=(6,1079)
1337	686	AV749844	184	Hs.26670	PAC clone RP3-515N1 from 22q11.2-q22 /cds=(0,791)
1453	687	BC003352	185	Hs.326456	hypothetical protein FLJ20030 (FLJ20030), mRNA /cds=(1,1239)
1298	688	AU135154	186	Hs.172028	a disintegrin and metalloproteinase domain 10 (ADAM10), mRNA /cds=(469,2715)
779	689	AF208850	187	Hs.82911	BM-008 mRNA, complete cds /cds=(341,844)
1602	690	D29805	188	Hs.198248	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1 (B4GALT1), mRNA /cds=(72,1268)
2867	691	NM_006098	189	Hs.5662	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1 (GNB2L1), mRNA /cds=(95,1048)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
2183	692	NM_001755	190	Hs.179881	core-binding factor, beta subunit (CBFB), transcript variant 2, mRNA /cds=(11,559)
3033	693	NM_007355	191	Hs.74335	heat shock 90kD protein 1, beta (HSPCB), mRNA /cds=(0,2174)
3692	694	W47229	192	Hs.173334	ELL-RELATED RNA POLYMERASE II, ELONGATION FACTOR (ELL2), mRNA /cds=(0,1922)
3741	695	X51345	193	Hs.198951	jun B proto-oncogene (JUNB), mRNA /cds=(253,1296)
5114	696	AI364677	194	Hs.368853	ESTs
5135	697	AI380594	195	Hs.231261	tf95h06.x1 cDNA, 3' end /clone=IMAGE:2107067 /clone_end=3'
2158	698	NM_001641	196	Hs.73722	APEX nuclease (multifunctional DNA repair enzyme) (APEX), mRNA /cds=(205,1161)
2369	699	NM_002786	197	Hs.82159	proteasome (prosome, macropain) subunit, alpha type, 1 (PSMA1), mRNA /cds=(105,896)
1676	700	J02621	198	Hs.251064	high-mobility group (nonhistone chromosomal) protein 14 (HMG14), mRNA /cds=(150,452)
3414	701	NM_019111	199	Hs.76807	major histocompatibility complex, class II, DR alpha (HLA-DRA), mRNA /cds=(26,790)
801	702	AF248966	200	Hs.183434	ATPase, H+ transporting, lysosomal (vacuolar proton pump) membrane sector associated protein M8-9 (APT6M8-9)
1605	703	D31767	201	Hs.75416	DAZ associated protein 2 (DAZAP2), mRNA
2974	704	NM_006839	202	Hs.78504	inner membrane protein, mitochondrial (mitofilin) (IMMT), mRNA /cds=(92,2368)
5333	705	AI581732	203	Hs.229918	ar74f03.x1 cDNA, 3' end /clone=IMAGE:2128349 /clone_end=3'
3411	706	NM_019059	204	Hs.274248	hypothetical protein FLJ20758 (FLJ20758), mRNA /cds=(464,1306)
2061	707	NM_001033	205	Hs.2934	ribonucleotide reductase M1 polypeptide (RRM1), mRNA /cds=(187,2565)
2361	708	NM_002719	206	Hs.171734	protein phosphatase 2, regulatory subunit B (B56), gamma isoform (PPP2R5C), mRNA /cds=(88,1632)
2553	709	NM_003791	207	Hs.75890	membrane-bound transcription factor protease, site 1 (MBTPS1), mRNA /cds=(496,3654)
2068	710	NM_001105	208	Hs.150402	activin A receptor, type I (ACVR1), mRNA /cds=(340,1869)
4153	711	BG179517	209	Hs.99093	chromosome 19, cosmid R28379 /cds=(0,633)
6179	712	BF940103	210	Hs.26136	hypothetical protein MGC14156 (MGC14156), mRNA /cds=(82,426)
668	713	AF061736	211	Hs.169895	ubiquitin-conjugating enzyme E2L 6 (UBE2L6), mRNA /cds=(47,508)
981	714	AK023680	212	Hs.17448	cDNA FLJ13618 fis, clone PLACE1010925 /cds=UNKNOWN
2102	715	NM_001295	213	Hs.301921	chemokine (C-C motif) receptor 1 (CCR1), mRNA /cds=(62,1129)
2560	716	NM_003811	214	Hs.1524	tumor necrosis factor (ligand) superfamily, member 9 (TNFSF9), mRNA /cds=(3,767)
3701	717	X02812	215	Hs.1103	transforming growth factor, beta 1 (TGFB1), mRNA /cds=(841,2016)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
4601	718	NM_002205	216	Hs.149609	integrin, alpha 5 (fibronectin receptor, alpha polypeptide) (ITGA5), mRNA /cds=(23,3172)
5468	719	AI818777	217	Hs.229990	wl11f10.x1 cDNA, 3' end /clone=IMAGE:2424619 /clone_end=3'
7641	720	NM_005892	218	Hs.100217	formin-like (FMNL), mRNA /cds=(39,1430)
8015	721	M26252	219	Hs.198281	pyruvate kinase, muscle (PKM2), mRNA
446	722	AB002377	220	Hs.32556	mRNA for KIAA0379 protein, partial cds /cds=(0,3180)
4359	723	AI381586	221	Hs.87908	Snf2-related CBP activator protein (SRCAP), mRNA /cds=(210,9125)
5034	724	BG760189	222	Hs.37617	602144947F1 cDNA, 5' end /clone=IMAGE:4308683 /clone_end=5'
4752	725	NM_006913	223	Hs.216354	ring finger protein 5 (RNF5), mRNA /cds=(0,542)
765	726	AF189011	224	Hs.49163	ribonuclease III (RN3) mRNA, complete cds /cds=(245,4369)
949	727	AK022834	225	Hs.58488	catenin (cadherin-associated protein), alpha-like 1 (CTNNAL1), mRNA /cds=(43,2247)
2398	728	NM_002878	226	Hs.125244	RAD51 (S. cerevisiae)-like 3 (RAD51L3), mRNA /cds=(124,993)
7445	729	BF899464	227	NA	IL5-MT0211-011200-317-f03 MT0211 cDNA, mRNA sequence
7748	730	AW452510	228	Hs.300479	UI-H-BW1-ame-a-12-0-UI.s1 cDNA, 3' end /clone=IMAGE:3069598 /clone_end=3'
2778	731	NM_005508	229	Hs.184926	chemokine (C-C motif) receptor 4 (CCR4), mRNA /cds=(182,1264)
3734	732	X16354	230	Hs.50964	mRNA for transmembrane carcinoembryonic antigen BGPa (formerly TM1-CEA) /cds=(72,1652)
4364	733	AA581115	231	Hs.291129	oe10d02.s1 cDNA /clone=IMAGE:1385475
2776	734	NM_005485	232	Hs.271742	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)-like 3 (ADPRTL3), mRNA /cds=(246,1847)
2826	735	NM_005816	233	Hs.142023	T cell activation, increased late expression (TACTILE), mRNA /cds=(928,2637)
3987	736	BG033294	234	Hs.118787	transforming growth factor, beta-induced, 68kD (TGFBI), mRNA /cds=(47,2098)
4501	737	K01566	235	Hs.69771	B-factor, properdin
6244	738	T25714	236	Hs.330530	ESTDIR309 cDNA, 3' end /clone=CDDIRX9
7346	739	NM_022873	237	Hs.265827	interferon, alpha-inducible protein (clone IFI-6-16) (G1P3), transcript variant 3, mRNA /cds=(107,523)
3827	740	X99699	238	Hs.139262	XIAP associated factor-1 (HSXIAPAF1), mRNA /cds=(0,953)
676	741	AF067519	239	Hs.307357	PITSLRE protein kinase beta SV1 isoform (CDC2L2) mRNA, complete cds /cds=(79,2412)
4988	742	BG387694	240	Hs.170980	cell cycle progression 2 protein (CPR2), mRNA /cds=(126,1691)
707	743	AF104032	241	Hs.184601	L-type amino acid transporter subunit LAT1 mRNA, complete cds /cds=(66,1589)
7351	744	NM_012177	242	Hs.272027	F-box only protein 5 (FBXO5), mRNA /cds=(61,1404)
1158	745	AL042370	243	Hs.79709	phosphotidylinositol transfer protein (PITPN), mRNA /cds=(216,1028)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
4489	746	BC009469	244	Hs.287797	mRNA for FLJ00043 protein, partial cds /cds=(0,4248)
4924	747	AA319163	245	Hs.424299	RPLP1 ;germinal
6275	748	AI393970	246	Hs.76239	hypothetical protein FLJ20608 (FLJ20608), mRNA /cds=(81,680)
3157	749	NM_014481	247	Hs.154149	Homo sapiens, apurinic/apyrimidinic endonuclease(APEX nuclease)-like 2 protein, clone MGC:1418 IMAGE:3139156, mRNA, complete cds
3338	750	NM_017774	248	Hs.306668	cDNA FLJ14089 fis, clone MAMMA1000257 /cds=UNKNOWN
3346	751	NM_017859	249	Hs.39850	hypothetical protein FLJ20517 (FLJ20517), mRNA /cds=(44,1690)
3544	752	R44202	250	Hs.240013	mRNA; cDNA DKFZp547A166 (from clone DKFZp547A166) /cds=UNKNOWN
4626	753	NM_002904	251	Hs.106061	RD RNA-binding protein (RDBP), mRNA /cds=(108,1250)
7339	754	AL133642	252	Hs.241471	mRNA; cDNA DKFZp586G1721 (from clone DKFZp586G1721); partial cds /cds=(0,669)
109	755	AF160973	253	Hs.258503	P53 inducible protein
285	756	NM_001972	254	Hs.99863	elastase 2, neutrophil (ELA2),
406	757	AA282774	255	NA	cDNA clone IMAGE:713136 5'
436	758	AB000115	256	Hs.75470	hypothetical protein, expressed in osteoblast (GS3686), mRNA /cds=(241,1482)
860	759	AJ277247	257	Hs.287369	interleukin 22 (IL22), mRNA /cds=(71,610)
1610	760	D38081	258	Hs.89887	thromboxane A2 receptor (TBXA2R), mRNA /cds=(991,2022)
2096	761	NM_001250	259	Hs.25648	tumor necrosis factor receptor superfamily, member 5 (TNFRSF5), mRNA /cds=(47,880)
5288	762	AI524266	260	Hs.230874	th11g12.x1 cDNA, 3' end /clone=IMAGE:2118022 /clone_end=3'
5534	763	AL573787	261	Hs.21732	AL573787 cDNA /clone=CS0DI055YM17-(3-prime)
7347	764	AK001503	262	Hs.265891	cDNA FLJ10641 fis, clone NT2RP2005748 /cds=UNKNOWN
279	765	X04430	263	Hs.93913	IFN-beta 2a mRNA for interferon-beta-2, T-cells, macrophages
349	766	AF480557	264	NA	142E4
1284	767	AL550229	265	Hs.271599	cDNA FLJ12347 fis, clone MAMMA1002298 /cds=UNKNOWN
1328	768	AV727063	266	Hs.245798	hypothetical protein DKFZp564I0422 (DKFZP564I0422), mRNA /cds=(510,1196)
1939	769	NM_000389	267	Hs.179665	cyclin-dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A), mRNA /cds=(75,569)
2186	770	NM_001761	268	Hs.1973	cyclin F (CCNF), mRNA /cds=(43,2403)
2364	771	NM_002741	269	Hs.2499	protein kinase C-like 1 (PRKCL1), mRNA /cds=(84,2912)
2400	772	NM_002880	270	Hs.279474	HSPC070 protein (HSPC070), mRNA /cds=(331,1581)
3146	773	NM_014373	271	Hs.97101	putative G protein-coupled receptor (GPCR150), mRNA /cds=(321,1337)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
3633	774	U53347	272	Hs.183556	solute carrier family 1 (neutral amino acid transporter), member 5 (SLC1A5), mRNA /cds=(590,2215)
3689	775	W19201	273	Hs.17778	neuropilin 2 (NRP2), mRNA /cds=(0,2780)
3695	776	W79598	274	Hs.163846	putative N6-DNA-methyltransferase (N6AMT1), mRNA /cds=(29,673)
4079	777	XM_001939	275	Hs.55468	H4 histone, family 2
4254	778	AI270476	276	Hs.270341	602307338F1 cDNA, 5' end /clone=IMAGE:4398848 /clone_end=5'
4316	779	AA992299	277	Hs.129332	ot53b06.s1 cDNA, 3' end /clone=IMAGE:1620467 /clone_end=3'
4394	780	AF044595	278	Hs.248078	lymphocyte-predominant Hodgkin's disease case #7 immunoglobulin heavy chain gene, variable region
4492	781	BI091076	279	Hs.127128	ok13e12.s1 cDNA, 3' end /clone=IMAGE:1507726 /clone_end=3'
4498	782	H13491	280	Hs.303450	yj15f02.r1 cDNA, 5' end /clone=IMAGE:148827 /clone_end=5'
4507	783	M55420	281	Hs.247930	IgE chain, last 2 exons
4778	784	NM_014271	282	Hs.241385	interleukin 1 receptor accessory protein-like 1 (IL1RAPL1), mRNA /cds=(510,2600)
5126	785	AI378091	283	Hs.369056	ESTs
5138	786	AI381601	284	Hs.159025	td05g03.x1 cDNA, 3' end /clone=IMAGE:2074804 /clone_end=3'
5347	787	AI634972	285	Hs.319825	602021477F1 cDNA, 5' end /clone=IMAGE:4156915 /clone_end=5'
5561	788	AW005376	286	Hs.173280	ws94a12.x1 cDNA, 3' end /clone=IMAGE:2505598 /clone_end=3'
5706	789	AW088500	287	Hs.389655	EST, Weakly similar to A35098 MHC class III histocompatibility antigen HLA-B-associated transcript 3
5735	790	AW195270	288	Hs.330019	xn67c04.x1 cDNA, 3' end /clone=IMAGE:2699526 /clone_end=3'
5836	791	AW296797	289	Hs.255579	UI-H-BW0-ajb-e-07-0-UI.s1 cDNA, 3' end /clone=IMAGE:2731117 /clone_end=3'
7438	792	BF827734	290	Hs.156766	ESTs
8009	793	M11233	291	Hs.79572	cathepsin D (lysosomal aspartyl protease) (CTSD), mRNA /cds=(2,1240)
1182	794	AL050218	292	Hs.15020	DNA sequence from clone 51J12 on chromosome 6q26-
3260	795	NM_016063	293	Hs.32826	CGI-130 protein (LOC51020), mRNA /cds=(63,575)
6702	796	BU678165	294	Hs.377992	479H5, not in ref seq, Rab geranylgeranyltransferase, alpha subunit (RABGGTA),
1186	797	AL050371	295	Hs.8128	phosphatidylserine decarboxylase (PISD), mRNA /cds=(223,1350)
330	798	NM_152545	296	Hs.335815	62C9, hypothetical protein FLJ31695
4107	799	XM_007156	297	Hs.159492	sacsin (SACS) gene, complete cds /cds=(76,11565)
4780	800	NM_014339	298	Hs.129751	interleukin 17 receptor (IL17R), mRNA /cds=(32,2632)
4821	801	NM_019598	299	Hs.159679	kallikrein 12 (KLK12), mRNA /cds=UNKNOWN
5091	802	AI081258	300	Hs.134590	oy67c11.x1 cDNA, 3' end /clone=IMAGE:1670900 /clone_end=3'

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
6000	803	AW468621	301	Hs.257743	he42e03.x1 cDNA, 3' end /clone=IMAGE:2921692 /clone_end=3'
2202	804	NM_001873	302	Hs.75360	carboxypeptidase E (CPE), mRNA /cds=(290,1720)
4886	805	NM_032839	303	Hs.11360	hypothetical protein FLJ14784 (FLJ14784), mRNA /cds=(133,1569)
3733	806	X16277	304	Hs.339703	zv26f06.r1 cDNA, 5' end /clone=IMAGE:754787 /clone_end=5'
4526	807	NM_000395	305	Hs.285401	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage) (CSF2RB), mRNA /cds=(28,2721)
3085	808	NM_013252	306	Hs.126355	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 5 (CLECSF5), mRNA /cds=(197,763)
343	809	129F10	307	NA	129F10, chromosome hit
1008	810	AK024331	308	Hs.287631	cDNA FLJ14269 fis, clone PLACE1003864 /cds=UNKNOWN
1911	811	NM_000195	309	Hs.83951	Hermansky-Pudlak syndrome (HPS), mRNA /cds=(206,2308)
7307	812	NM_030756	310	Hs.173638	transcription factor 7-like 2 (T-cell specific, HMG-box) (TCF7L2), mRNA /cds=(307,2097)
150	813	M26683	311	Hs.303649	interferon gamma treatment inducible mRNA Monocytes
399	814	AA214691	312	Hs.111377	LOC286530 hypothetical protein LOC286530
599	815	AB049113	313	Hs.82113	dUTP pyrophosphatase (DUT), mRNA /cds=(29,523)
1129	816	AK026819	314	Hs.20242	hypothetical protein FLJ12788 (FLJ12788), mRNA /cds=(9,866)
1717	817	L21961	315	Hs.181125	Homo sapiens, clone MGC:12849 IMAGE:4308973, mRNA, complete cds /cds=(24,725)
2101	818	NM_001278	316	Hs.306440	mRNA; cDNA DKFZp566L084 (from clone DKFZp566L084) /cds=UNKNOWN
2290	819	NM_002385	317	Hs.69547	myelin basic protein (MBP), mRNA /cds=(10,570)
2736	820	NM_005121	318	Hs.11861	thyroid hormone receptor-associated protein, 240 kDa subunit (TRAP240), mRNA /cds=(77,6601)
3018	821	NM_007220	319	Hs.283646	carbonic anhydrase VB, mitochondrial (CA5B), nuclear gene encoding mitochondrial protein, mRNA /cds=(137,1090)
3068	822	NM_012381	320	Hs.74420	origin recognition complex, subunit 3 (yeast homolog)-like (ORC3L), mRNA /cds=(26,2161)
3128	823	NM_014225	321	Hs.173902	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65
3965	824	BF966028	322	Hs.5324	hypothetical protein (CL25022), mRNA /cds=(157,1047)
4450	825	AL157438	323	Hs.66151	mRNA; cDNA DKFZp434A115 (from clone DKFZp434A115) /cds=UNKNOWN
4677	826	NM_004488	324	Hs.73734	glycoprotein V (platelet) (GP5), mRNA /cds=(270,1952)
4753	827	NM_006929	325	Hs.153299	DOM-3 (C. elegans) homolog Z (DOM3Z), transcript variant 2, mRNA /cds=(129,1319)
4841	828	NM_021976	326	Hs.79372	retinoid X receptor, beta (RXRB), mRNA /cds=(179,1780)
4891	829	T93822	327	Hs.294092	EST375308 cDNA

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
5284	830	AI524202	328	Hs.171122	th10d11.x1 cDNA, 3' end /clone=IMAGE:2117877 /clone_end=3'
5359	831	AI684022	329	Hs.90744	proteasome (prosome, macropain) 26S subunit, non-ATPase, 11 (PSMD11), mRNA /cds=(0,1268)
5965	832	AW452545	330	Hs.257582	UI-H-BW1-ame-d-12-0-UI.s1 cDNA, 3' end /clone=IMAGE:3069742 /clone_end=3'
6434	833	NM_153341	331	Hs.64239	DNA sequence from clone RP5-1174N9 on chromosome 1p34.1-35.3.
7175	834	BF698885	332	Hs.5890	hypothetical protein FLJ23306 (FLJ23306), mRNA /cds=(562,930)
1884	835	NM_000073	333	Hs.2259	CD3G antigen, gamma polypeptide (TiT3 complex) (CD3G), mRNA /cds=(37,585)
2683	836	NM_004761	334	Hs.170160	RAB2, member RAS oncogene family-like (RAB2L), mRNA /cds=(0,2333)
3240	837	NM_015898	335	Hs.104640	HIV-1 inducer of short transcripts binding protein (FBI1), mRNA /cds=(0,1754)
4781	838	NM_014348	336	Hs.296429	similar to rat integral membrane glycoprotein POM121 (POM121L1), mRNA /cds=(0,1286)
4950	839	AW500534	337	Hs.145668	fmfc5 cDNA /clone=CR6-21
5076	840	AA765569	338	Hs.104157	EST380899 cDNA
5092	841	AI084553	339	Hs.105621	HNC29-1-B1.R cDNA
5264	842	AI523617	340	Hs.171098	tg95b03.x1 cDNA, 3' end /clone=IMAGE:2116493 /clone_end=3'
5514	843	AI969716	341	Hs.13034	hv63f09.x1 cDNA, 3' end /clone=IMAGE:3178121 /clone_end=3'
7301	844	NM_002076	342	Hs.164036	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID) (GNS), mRNA /cds=(87,1745)
5387	845	AI760224	343	Hs.26873	wh62g06.x1 cDNA, 3' end /clone=IMAGE:2385370 /clone_end=3'
5530	846	AL565736	344	Hs.181165	eukaryotic translation elongation factor 1 alpha 1 (EEF1A1), mRNA /cds=(53,1441)
7330	847	NM_004900	345	Hs.226307	phorbolin (similar to apolipoprotein B mRNA editing protein) (DJ742C19.2), mRNA /cds=(79,651)
5083	848	AI031624	346	Hs.238954	602637935F1 cDNA, 5' end /clone=IMAGE:4765448 /clone_end=5'
6130	849	BF059133	347	Hs.144583	Homo sapiens, clone IMAGE:3462401, mRNA, partial cds /cds=(0,153)
555	850	AB036432	348	Hs.184	advanced glycosylation end product-specific receptor (AGER), mRNA /cds=(0,1214)
3547	851	R64054	349	Hs.208603	7f01d11.x1 cDNA, 3' end /clone=IMAGE:3293397 /clone_end=3'
1849	852	M81601	350	Hs.153179	fatty acid binding protein 5 (psoriasis-associated) (FABP5), mRNA /cds=(48,455)
1390	853	AY004255	351	Hs.238990	Homo sapiens, Similar to cyclin-dependent kinase inhibitor 1B (p27, Kip1)
4604	854	NM_002258	352	Hs.169824	killer cell lectin-like receptor subfamily B, member 1 (KLRB1), mRNA /cds=(60,737)
1750	855	M11124	353	Hs.198253	major histocompatibility complex, class II, DQ alpha 1 (HLA-DQA1), mRNA /cds=(43,810)
4400	856	AF073705	354	Hs.247721	clone mcg53-54 immunoglobulin lambda light chain variable region 4a mRNA, partial cds /cds=(0,324)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
855	857	AJ271326	355	Hs.135187	unc93 (C.elegans) homolog B (UNC93B), mRNA /cds=(41,1834)
6566	858	NM_138391	356	Hs.17481	mRNA; cDNA DKFZp434G2415 (from clone DKFZp434G2415) /cds=UNKNOWN
3825	859	X97324	357	Hs.3416	adipose differentiation-related protein (ADFP), mRNA /cds=(0,1313)
2895	860	NM_006289	358	Hs.18420	talin 1 (TLN1), mRNA /cds=(126,7751)
4631	861	NM_002935	359	Hs.73839	ribonuclease, RNase A family, 3 (eosinophil cationic protein) (RNASE3), mRNA /cds=(63,545)
3832, 8069	862	Y00345	360	Hs.172182	poly(A)-binding protein, cytoplasmic 1 (PABPC1), mRNA /cds=(502,2403)
5531	863	AL567986	361	Hs.77393	farnesyl diphosphate synthase
4149	864	NM_000311	362	Hs.74621	prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome)
3305	865	NM_016523	363	Hs.183125	killer cell lectin-like receptor subfamily F, member 1 (KLRF1), mRNA /cds=(64,759)
5074	866	AA701193	364	Hs.431104	EST, Weakly similar to HA21_HUMAN HLA class II histocompatibility antigen, DQ(1) alpha chain precursor (DC-4 alpha chain)
8095	867	AK026594	365	Hs.251653	tubulin, beta, 2 (TUBB2), mRNA /cds=(0,1337)
5607	868	AW063509	366	Hs.279105	TN1012 cDNA, 3' end /clone_end=3'
3541	869	R14692	367	Hs.170222	Na+/H+ exchanger NHE-1 isoform [human, heart, mRNA, 4516 nt] /cds=(577,3024)
2386	870	NM_002831	368	Hs.63489	protein tyrosine phosphatase, non-receptor type 6 (PTPN6), mRNA /cds=(144,1931)
1535	871	BE868389	369	Hs.179703	tripartite motif protein 14 (TRIM14), mRNA
328	872	AK090404	370	Hs.98531	53G7, FLJ00290 protein
1001	873	AK024202	371	Hs.289088	heat shock 90kD protein 1, alpha (HSPCA), mRNA /cds=(60,2258)
1003	874	AK024240	372	Hs.24115	cDNA FLJ14178 fis, clone NT2RP2003339 /cds=UNKNOWN
1025	875	AK024756	373	Hs.12293	hypothetical protein FLJ21103 (FLJ21103), mRNA /cds=(88,1143)
1035	876	AK024969	374	Hs.166254	hypothetical protein DKFZp566I133 (DKFZP566I133), mRNA /cds=(133,1353)
1227	877	AL136542	375	Hs.322456	hypothetical protein DKFZp761D0211 (DKFZP761D0211), mRNA /cds=(164,1822)
3249	878	NM_015995	376	Hs.7104	mRNA; cDNA DKFZp761P06121 (from clone DKFZp761P06121) /cds=UNKNOWN
2060	879	NM_001032	377	Hs.539	ribosomal protein S29 (RPS29), mRNA /cds=(30,200)
4902	880	X58397	378	Hs.81220	CLL-12 transcript of unrearranged immunoglobulin V(H)5 gene /cds=(39,425)
5782	881	AW293895	379	Hs.255249	UI-H-BW0-ain-f-10-0-UI.s1 cDNA, 3' end /clone=IMAGE:2729995 /clone_end=3'
7626	882	NM_003128	380	Hs.324648	cDNA FLJ13700 fis, clone PLACE2000216, highly similar to SPECTRIN BETA CHAIN, BRAIN /cds=UNKNOWN
1195	883	AL109669	381	Hs.172803	mRNA full length insert cDNA clone EUROIMAGE 31839 /cds=UNKNOWN

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
8111	884	AI307808	382	Hs.238797	602081661F1 cDNA, 5' end /clone=IMAGE:4245999 /clone_end=5'
805	885	AF261087	383	Hs.174131	ribosomal protein L6 (RPL6), mRNA /cds=(26,892)
2330	886	NM_002546	384	Hs.81791	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) (TNFRSF11B), mRNA /cds=(94,1299)
3053	887	NM_012237	385	Hs.44017	sirtuin (silent mating type information regulation 2, S.cerevisiae, homolog) 2 (SIRT2), transcript variant 1, mRNA /cds=(200,1369)
3779	888	X68060	386	Hs.75248	topoisomerase (DNA) II beta (180kD) (TOP2B), mRNA /cds=(0,4865)
5353	889	AI660405	387	Hs.111941	qd92a04.x1 cDNA, 3' end /clone=IMAGE:1736910 /clone_end=3'
5442	890	AI798114	388	Hs.210307	wh81c01.x1 cDNA, 3' end /clone=IMAGE:2387136 /clone_end=3'
5690	891	AW075948	389	Hs.265634	xa82b03.x1 cDNA, 3' end /clone=IMAGE:2573261 /clone_end=3'
5791	892	AW294681	390	Hs.255336	UI-H-BW0-ail-g-10-0-UI.s1 cDNA, 3' end /clone=IMAGE:2729683 /clone_end=3'
6239	893	R40823	391	Hs.108082	602068988F1 cDNA, 5' end /clone=IMAGE:4067972 /clone_end=5'
6260	894	AA806222	392	Hs.111554	ADP-ribosylation factor-like 7 (ARL7), mRNA /cds=(14,592)
6273	895	AI380390	393	Hs.158976	UI-H-BI2-ahi-a-03-0-UI.s1 cDNA, 3' end /clone=IMAGE:2726692 /clone_end=3'
6351	896	BF435621	394	Hs.293476	hypothetical protein FKSG44 (FKSG44), mRNA /cds=(126,1520)
7171	897	AK025781	395	Hs.5076	cDNA: FLJ22128 fis, clone HEP19543 /cds=UNKNOWN
8053	898	X06323	396	Hs.79086	mitochondrial ribosomal protein L3 (MRPL3), mRNA /cds=(76,1122)
8065	899	X72841	397	Hs.31314	retinoblastoma-binding protein 7 (RBBP7), mRNA /cds=(287,1564)
78	900	NM_001015	398	Hs.182740	ribosomal protein S11 (RPS11), mRNA /cds=(15,4
174	901	J02931	399	Hs.62192	placental tissue factor (two forms) mRNA, complete cd
252	902	D49950	400	Hs.83077	for interferon-gamma inducing activated macrophages
255	903	NM_001772	401	Hs.83731	CD33 antigen (gp67) (CD33), mRNA.
824	904	AF307339	402	Hs.47783	B aggressive lymphoma gene (BAL), mRNA /cds=(228,2792)
1022	905	AK024597	403	Hs.10362	cDNA: FLJ20944 fis, clone ADSE01780
1155	906	AK027260	404	Hs.152925	mRNA for KIAA1268 protein, partial cds /cds=(0,3071)
1270	907	AL360190	405	Hs.318501	stimulated trans-acting factor (50 kDa) (STAF50), mRNA /cds=(122,1450)
1301	908	AV689330	406	Hs.189402	Similar to RIKEN cDNA 2210009G21 gene, clone IMAGE:4807023
1443	909	BC002796	407	Hs.46446	lymphoblastic leukemia derived sequence 1 (LYL1), mRNA /cds=(0,803)
1561	910	BE899595	408	NA	cDNA clone IMAGE:3952215 5'
1695	911	K02766	409	Hs.1290	complement component 9 (C9), mRNA /cds=(4,1683)
2070	912	NM_001111	410	Hs.7957	adenosine deaminase, RNA-specific (ADAR), transcript variant ADAR-a, mRNA /cds=(187,3867)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
2140	913	NM_001549	411	Hs.181874	interferon-induced protein with tetratricopeptide repeats 4 (IFIT4), mRNA /cds=(61,1533)
2190	914	NM_001778	412	Hs.901	CD48 antigen (B-cell membrane protein) (CD48), mRNA /cds=(36,767)
2312	915	NM_002463	413	Hs.926	myxovirus (influenza) resistance 2, homolog of murine (MX2), mRNA /cds=(104,2251)
2367	916	NM_002759	414	Hs.274382	protein kinase, interferon-inducible double stranded RNA dependent (PRKR), mRNA /cds=(435,2090)
2593	917	NM_004031	415	Hs.166120	interferon regulatory factor 7 (IRF7), transcript variant d, mRNA /cds=(335,1885)
2979	918	NM_006865	416	Hs.113277	leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 3 (LILRA3), mRNA /cds=(62,1381)
3097	919	NM_013352	417	Hs.58636	squamous cell carcinoma antigen recognized by T cell (SART-2), mRNA /cds=(149,3025)
3114	920	NM_014065	418	Hs.279040	HT001 protein (HT001), mRNA /cds=(241,1203)
3328	921	NM_017523	419	Hs.139262	XIAP associated factor-1 (HSXIAPAF1), mRNA /cds=(0,953)
3454	922	NM_021105	420	Hs.198282	phospholipid scramblase 1 (PLSCR1), mRNA /cds=(256,1212)
4096	923	XM_005543	421	Hs.234642	aquaporin 3 (AQP3), mRNA /cds=(64,942)
7264	924	NM_009587	422	Hs.81337	lectin, galactoside-binding, soluble, 9 (galectin 9) (LGALS9), transcript variant long, mRNA /cds=(56,1123)
4060	925	BG505271	423	Hs.86437	602411368F1 cDNA, 5' end /clone=IMAGE:4540096 /clone_end=5'
6122	926	BE965319	424	Hs.286754	601659229R1 cDNA, 3' end /clone=IMAGE:3895783 /clone_end=3'
2481	927	NM_003264	425	Hs.63668	toll-like receptor 2 (TLR2), mRNA /cds=(129,2483)
379	928	BU540019	426	NA	485A6, EST
1318	929	AV719442	427	Hs.256959	AV719442 cDNA, 5' end /clone=GLCBNA01
2005	930	NM_000879	428	Hs.2247	interleukin 5 (colony-stimulating factor, eosinophil) (IL5), mRNA /cds=(44,448)
2208	931	NM_001916	429	Hs.289271	cytochrome c-1 (CYC1), mRNA /cds=(8,985)
2309	932	NM_002460	430	Hs.82132	interferon regulatory factor 4 (IRF4), mRNA /cds=(105,1460)
2434	933	NM_002994	431	Hs.89714	small inducible cytokine subfamily B (Cys-X-Cys), member 5
2994	934	NM_007015	432	Hs.97932	chondromodulin I precursor (CHM-I), mRNA /cds=(0,1004)
3332	935	NM_017644	433	Hs.246875	hypothetical protein FLJ20059 (FLJ20059), mRNA /cds=(25,1290)
3757	936	X57025	434	Hs.85112	IGF-I mRNA for insulin-like growth factor I
3960	937	BF892532	435	Hs.38664	IL0-MT0152-061100-501-e04 cDNA
3984	938	BG028577	436	Hs.279009	matrix Gla protein (MGP), mRNA /cds=(46,357)
4403	939	AF116909	437	Hs.167827	clone HH419 unknown mRNA /cds=(189,593)
4447	940	AL136842	438	Hs.260024	mRNA; cDNA DKFZp434A0530 (from clone DKFZp434A0530); complete cds /cds=(968,1732)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
4463	941	AW327360	439	Hs.250605	dq02e11.x1 cDNA, 5' end /clone=IMAGE:2846685 /clone_end=5'
5295	942	AI538420	440	Hs.231292	td06a03.x1 cDNA, 3' end /clone=IMAGE:2074828 /clone_end=3'
5452	943	AI805144	441	NA	EST
5642	944	AW064160	442	Hs.279141	SP0594 cDNA, 3' end /clone_end=3'
5699	945	AW078847	443	Hs.244816	xb18g07.x1 cDNA, 3' end /clone=IMAGE:2576700 /clone_end=3'
5746	946	AW236252	444	Hs.253747	xn71g08.x1 cDNA, 3' end /clone=IMAGE:2699966 /clone_end=3'
5841	947	AW297026	445	Hs.255600	UI-H-BW0-ajf-e-06-0-UI.s1 cDNA, 3' end /clone=IMAGE:2731499 /clone_end=3'
7608	948	NM_016095	446	Hs.108196	HSPC037 protein (LOC51659), mRNA /cds=(78,635)
877	949	AK000575	447	Hs.279581	hypothetical protein FLJ20568 (FLJ20568), mRNA /cds=(6,422)
2311	950	NM_002462	448	Hs.76391	myxovirus (influenza) resistance 1, homolog of murine (interferon-inducible protein p78) (MX1), mRNA /cds=(345,2333)
2566	951	NM_003841	449	Hs.119684	tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain (TNFRSF10C), mRNA /cds=(29,928)
2693	952	NM_004834	450	Hs.3628	mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4), mRNA /cds=(79,3576)
3098	953	NM_013368	451	Hs.169138	RPA-binding trans-activator (RBT1), mRNA /cds=(291,881)
3723	954	X12451	452	Hs.78056	cathepsin L (CTSL), mRNA /cds=(288,1289)
3847	955	Y13936	453	Hs.17883	protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform (PPM1G), mRNA /cds=(24,1664)
4324	956	AW190635	454	Hs.15200	EST379783 cDNA
4373	957	AI378123	455	Hs.327454	tc80e02.x1 cDNA, 3' end /clone=IMAGE:2072474 /clone_end=3'
4429	958	AJ275405	456	Hs.272362	partial IGVL1 gene for immunoglobulin lambda light chain V region
5075	959	AA729508	457	Hs.307486	nx54a03.s1 cDNA /clone=IMAGE:1266028
5490	960	AI865603	458	Hs.341208	wk47g03.x1 cDNA, 3' end /clone=IMAGE:2418580 /clone_end=3'
6958	961	NM_080612	459	Hs.102630	128F5, GRB2-associated binding protein 3 (GAB3),
183	962	NM_014086	460	Hs.6975	PRO1073 protein (PRO1073),
1712	963	L11695	461	Hs.220	transforming growth factor, beta receptor I (activin A receptor type II-like kinase, 53kD) (TGFBR1), mRNA /cds=(76,1587)
2435	964	NM_002995	462	Hs.3195	small inducible cytokine subfamily C, member 1 (lymphotactin) (SCYC1), mRNA /cds=(20,364)
3971	965	BF968963	463	Hs.5064	602490910F1 cDNA, 5' end /clone=IMAGE:4619835 /clone_end=5'

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
4024	966	BG286649	464	Hs.323950	zinc finger protein 6 (CMPX1) (ZNF6), mRNA /cds=(1265,3361)
4773	967	NM_014148	465	Hs.278944	HSPC048 protein (HSPC048), mRNA /cds=(87,419)
6140	968	BF195579	466	Hs.232257	RST2302 cDNA
6164	969	BF509758	467	Hs.144265	UI-H-BI4-apg-d-04-0-UI.s1 cDNA, 3' end /clone=IMAGE:3087390 /clone_end=3'
724	970	AF118274	468	Hs.128740	DNb-5 mRNA, partial cds /cds=(0,1601)
2729	971	NM_005082	469	Hs.1579	zinc finger protein 147 (estrogen-responsive finger protein) (ZNF147), mRNA /cds=(39,1931)
4267	972	AA576947	470	Hs.188886	nm82b04.s1 cDNA, 3' end /clone=IMAGE:1074703 /clone_end=3'
5073	973	AA628833	471	NA	EST
5345	974	AI631850	472	Hs.340604	wa36h07.x1 cDNA, 3' end /clone=IMAGE:2300221 /clone_end=3'
5564	975	AW006867	473	Hs.231987	602320903F1 cDNA, 5' end /clone=IMAGE:4424065 /clone_end=5'
1856	976	M94046	474	Hs.7647	MYC-associated zinc finger protein (purine-binding transcription factor) (MAZ), mRNA /cds=(91,1584)
449	977	AB007861	475	Hs.118047	602971981F1 cDNA, 5' end /clone=IMAGE:5111324 /clone_end=5'
670	978	AF061944	476	Hs.432900	PRKWNK1 protein kinase, lysine deficient 1
1237	979	AL136797	477	Hs.273294	mRNA; cDNA DKFZp434N031 (from clone DKFZp434N031); complete cds /cds=(18,3608)
1616	980	D42040	478	Hs.75243	bromodomain-containing 2 (BRD2), mRNA /cds=(1701,4106)
4268	981	AI089359	479	Hs.130232	qb05h03.x1 cDNA, 3' end /clone=IMAGE:1695413 /clone_end=3'
4690	982	NM_004776	480	Hs.107526	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 5 (B4GALT5), mRNA /cds=(112,1278)
4828	983	NM_020428	481	Hs.105509	cDNA FLJ14613 fis, clone NT2RP1001113, highly similar to CTL2 gene /cds=UNKNOWN
4829	984	NM_020530	482	Hs.248156	oncostatin M (OSM), mRNA /cds=(0,758)
300	985	NM_003321	483	Hs.12084	Tu translation elongation factor, mitochondrial (TUFM)
1562	986	BE901218	484	Hs.285122	Homo sapiens, hypothetical protein FLJ21839, clone MGC:2851 IMAGE:2967512, mRNA, complete cds /cds=(444,2618)
5112	987	AI361733	485	Hs.157811	qz24b02.x1 cDNA, 3' end /clone=IMAGE:2027787 /clone_end=3'
1104	988	AK026410	486	Hs.236449	hypothetical protein FLJ22757 (FLJ22757), mRNA /cds=(92,2473)
4017	989	BG254292	487	NA	cDNA clone IMAGE:4477042 5'
4565	990	NM_001504	488	Hs.198252	G protein-coupled receptor 9 (GPR9), mRNA /cds=(68,1174)
6121	991	BE964596	489	Hs.184052	PP1201 protein (PP1201), mRNA /cds=(75,1010)
458	992	AB011098	490	Hs.59403	serine palmitoyltransferase, long chain base subunit 2 (SPTLC2), mRNA /cds=(188,1876)
1519	993	BE745645	491	Hs.127951	hypothetical protein FLJ14503 (FLJ14503), mRNA /cds=(19,2217)

Table 2E.

OID	SEQ ID 50mer	ACC	SEQ ID Full length	HS	Gene
5477	994	AI827950	492	Hs.342617	ha15h10.x1 cDNA, 3' end /clone=IMAGE:2873827 /clone_end=3'
5522	995	AL521097	493	Hs.13144	HSPC160 protein (HSPC160), mRNA /cds=(53,514)
6026	996	BE222032	494	Hs.128675	hr61g11.x1 cDNA, 3' end /clone=IMAGE:3133028 /clone_end=3'
4283	997	AA516406	495	Hs.180201	hypothetical protein FLJ20671 (FLJ20671), mRNA /cds=(72,494)
861	998	AJ277832	496	Hs.56247	mRNA for inducible T-cell co-stimulator (ICOS gene) /cds=(67,666)
5550	999	AV653169	497	Hs.5897	cDNA FLJ13388 fis, clone PLACE1001168 /cds=UNKNOWN
1813	1000	M36820	498	Hs.75765	GRO2 oncogene (GRO2), mRNA /cds=(74,397)
3242	1001	NM_015919	499	Hs.145956	zinc finger protein mRNA, complete cds /cds=(1073,3133)
5128	1002	AI378109	500	Hs.283438	7f19b03.x1 cDNA, 3' end /clone=IMAGE:3295085 /clone_end=3'
5195	1003	AI436418	501	Hs.369051	ESTs, Weakly similar to VAM5_HUMAN Vesicule-associated membrane protein 5 (VAMP-5) (Myobrevin) (HSPC191)
3497	1004	NM_022488	502	Hs.26367	PC3-96 protein (PC3-96), mRNA /cds=(119,586)

Table 3

Disease Classification	Disease/Patient Group
Endocrine Disease	Diabetes Mellitus I and II Thyroiditis Autoimmune polyglandular syndrome Autoimmune oophoritis Autoimmune hypophysitis Addisson's Disease
Inflammatory/Rheumatic	Rheumatoid Arthritis Systemic Lupus Erythematosis Sjogrens Disease CREST syndrome Scleroderma Ankylosing Spondylitis Crohn's Ulcerative Colitis Primary Sclerosing Cholangitis
Inflammatory/Rheumatic	Appendicitis Diverticulitis Primary Biliary Sclerosis Wegener's Granulomatosis Polyarteritis nodosa Whipple's Disease Psoriasis Microscopic Polyanngiitis Takayasu's Disease Kawasaki's Disease Autoimmune hepatitis Asthma Churg-Strauss Disease Beurger's Disease Raynaud's Disease Cholecystitis Sarcoidosis Asbestosis Pneumoconioses Otic inflammatory disease Ophthalmic inflammatory disease Antinflammatory drug use
Neurological Disease	Alzheimer's Dementia Pick's Disease Multiple Sclerosis Guillain Barre Syndrome Post-viral neuropathies Peripheral Neuropathy

Table 4: Real-time PCR assay chemistries. Various combinations of reporter and quencher dyes are useful for real-time PCR assays.

Reporter	Quencher
FAM	TAMRA
	BHQ1
TET	TAMRA
	BHQ1
JOE	TAMRA
	BHQ1
HEX	TAMRA
	BHQ1
VIC	TAMRA
	BHQ1
ROX	BHQ2
TAMRA	BHQ2

We claim:

1. A method of diagnosing or monitoring auto immune and chronic inflammatory diseases in a patient, comprising detecting the expression level of one or more genes in said patient to diagnose or monitor auto immune and chronic inflammatory diseases in said patient wherein said one or more genes comprise a nucleotide sequence selected from the group consisting of SEQ ID NO:503, SEQ ID NO:505, SEQ ID NO:506, SEQ ID NO:508, SEQ ID NO:509, SEQ ID NO:510, SEQ ID NO:511, SEQ ID NO:512, SEQ ID NO:513, SEQ ID NO:514, SEQ ID NO:515, SEQ ID NO:516, SEQ ID NO:517, SEQ ID NO:518, SEQ ID NO:519, SEQ ID NO:520, SEQ ID NO:521, SEQ ID NO:522, SEQ ID NO:523, SEQ ID NO:524, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:540, SEQ ID NO:541, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, SEQ ID NO:545, SEQ ID NO:546, SEQ ID NO:547, SEQ ID NO:548, SEQ ID NO:549, SEQ ID NO:550, SEQ ID NO:551, SEQ ID NO:552, SEQ ID NO:553, SEQ ID NO:554, SEQ ID NO:555, SEQ ID NO:556, SEQ ID NO:557, SEQ ID NO:558, SEQ ID NO:559, SEQ ID NO:560, SEQ ID NO:561, SEQ ID NO:562, SEQ ID NO:563, SEQ ID NO:564, SEQ ID NO:565, SEQ ID NO:566, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:569, SEQ ID NO:570, SEQ ID NO:571, SEQ ID NO:572, SEQ ID NO:573, SEQ ID NO:574, SEQ ID NO:575, SEQ ID NO:576, SEQ ID NO:577, SEQ ID NO:578, SEQ ID NO:579, SEQ ID NO:580, SEQ ID NO:581, SEQ ID NO:582, SEQ ID NO:583, SEQ ID NO:584, SEQ ID NO:585, SEQ ID NO:586, SEQ ID NO:587, SEQ ID NO:588, SEQ ID NO:589, SEQ ID NO:590, SEQ ID NO:591, SEQ ID NO:592, SEQ ID NO:593, SEQ ID NO:594, SEQ ID NO:595, SEQ ID NO:596, SEQ ID NO:597, SEQ ID NO:598, SEQ ID NO:599, SEQ ID NO:600, SEQ ID NO:601, SEQ ID NO:602, SEQ ID NO:604, SEQ ID NO:605, SEQ ID NO:606, SEQ ID NO:607, SEQ ID NO:608, SEQ ID NO:609, SEQ ID NO:610, SEQ ID NO:611, SEQ ID NO:612, SEQ ID NO:613, SEQ ID NO:614, SEQ ID NO:615, SEQ ID NO:616, SEQ ID NO:617, SEQ ID NO:618, SEQ ID NO:619, SEQ ID NO:620, SEQ ID NO:621, SEQ ID NO:622, SEQ ID NO:623, SEQ ID NO:624, SEQ ID NO:625, SEQ ID NO:626, SEQ ID NO:627, SEQ ID NO:628, SEQ ID NO:629, SEQ ID NO:630, SEQ ID NO:631, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:634, SEQ ID NO:635, SEQ ID NO:636, SEQ ID NO:637, SEQ ID NO:638, SEQ ID NO:639, SEQ ID NO:640, SEQ ID NO:641, SEQ ID NO:642, SEQ ID NO:643, SEQ ID NO:644, SEQ ID NO:645, SEQ ID NO:646, SEQ ID NO:647, SEQ ID NO:648, SEQ ID NO:649, SEQ ID NO:650, SEQ ID NO:651, SEQ ID NO:652, SEQ ID NO:653, SEQ ID NO:654, SEQ ID NO:655, SEQ ID NO:656, SEQ ID NO:657, SEQ ID NO:658, SEQ ID NO:659, SEQ ID NO:660, SEQ ID NO:661, SEQ ID NO:662, SEQ ID NO:663, SEQ ID NO:664, SEQ ID NO:665, SEQ ID NO:666, SEQ ID NO:667, SEQ ID NO:668, SEQ ID NO:669, SEQ ID NO:670, SEQ ID NO:671, SEQ ID NO:672, SEQ ID NO:673, SEQ ID NO:674, SEQ ID NO:675, SEQ ID NO:676, SEQ ID NO:677, SEQ ID NO:678, SEQ ID NO:679, SEQ ID NO:680, SEQ ID

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2. The method of claim 1, further comprising detecting the expression level of one or more additional genes in said patient to diagnose or monitor auto immune and chronic inflammatory diseases in a patient, wherein said one or more additional genes comprise a nucleotide sequence selected from the group consisting of: SEQ ID NO:504, SEQ ID NO:507, SEQ ID NO:603, SEQ ID NO:740, SEQ ID NO:811, SEQ ID NO:812, SEQ ID NO:854, SEQ ID NO:867, SEQ ID NO:912, SEQ ID NO:922, SEQ ID NO:950, SEQ ID NO:971.
3. The method of claim 1 comprising detecting the expression level of at least two of said genes.
4. The method of claim 1 comprising detecting the expression level of at least ten of said genes.
5. The method of claim 1 comprising detecting the expression level of at least one hundred of said genes.
6. The method of claim 1 comprising detecting the expression level of all said genes.
7. The method of claim 1, wherein said auto immune and chronic inflammatory diseases is selected from the group consisting of: Rheumatoid Arthritis, Cholecystitis, Systemic Lupus Erythematosis, Sjogrens Disease, CREST syndrome, Scleroderma, Ankylosing Spondylitis, Crohn's, Ulcerative Colitis, Primary Sclerosing Cholangitis, Appendicitis, Diverticulitis, Primary Biliary Sclerosis, Wegener's Granulomatosis, Polyarteritis nodosa, Whipple's Disease, Psoriasis, Microscopic Polyanngiitis, Takayasu's Disease, Kawasaki's Disease,

Autoimmune hepatitis, Asthma, Churg-Strauss Disease, Beurger's Disease, Raynaud's Disease, and Cholecystitis.

8. The method of claim 1 wherein said diseases is Systemic Lupus Erythematosis.
9. The method of claim 1 wherein said diseases is Rheumatoid Arthritis.
10. The method of claim 1 wherein said expression level is detected by measuring the RNA level expressed by said one or more genes.
11. The method of claim 10, further including isolating RNA from said patient prior to detecting said RNA level expressed by said one or more genes.
12. The method of claim 10 wherein said RNA level is detected by PCR.
13. The method of claim 12 wherein said PCR uses primers consisting of nucleotide sequences selected from the group consisting of [SEQ ID NO: Z – primer pairs].
14. The method of claim 10 wherein said RNA level is detected by hybridization.
15. The method of claim 10 wherein said RNA level is detected by hybridization to an oligonucleotide.
16. The method of claim 15 wherein said oligonucleotide consists of a nucleotide sequence selected from the group consisting of SEQ ID NO:503, SEQ ID NO:504, SEQ ID NO:505, SEQ ID NO:506, SEQ ID NO:507, SEQ ID NO:508, SEQ ID NO:509, SEQ ID NO:510, SEQ ID NO:511, SEQ ID NO:512, SEQ ID NO:513, SEQ ID NO:514, SEQ ID NO:515, SEQ ID NO:516, SEQ ID NO:517, SEQ ID NO:518, SEQ ID NO:519, SEQ ID NO:520, SEQ ID NO:521, SEQ ID NO:522, SEQ ID NO:523, SEQ ID NO:524, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:540, SEQ ID NO:541, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, SEQ ID NO:545, SEQ ID NO:546, SEQ ID NO:547, SEQ ID NO:548, SEQ ID NO:549, SEQ ID NO:550, SEQ ID NO:551, SEQ ID NO:552, SEQ ID NO:553, SEQ ID NO:554, SEQ ID NO:555, SEQ ID NO:556, SEQ ID NO:557, SEQ ID NO:558, SEQ ID NO:559, SEQ ID NO:560, SEQ ID NO:561, SEQ ID NO:562, SEQ ID NO:563, SEQ ID NO:564, SEQ ID NO:565, SEQ ID NO:566, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:569, SEQ ID NO:570, SEQ ID NO:571, SEQ ID NO:572, SEQ ID NO:573, SEQ ID NO:574, SEQ ID NO:575, SEQ ID NO:576, SEQ ID NO:577, SEQ ID NO:578, SEQ ID NO:579, SEQ ID NO:580, SEQ ID NO:581, SEQ ID NO:582, SEQ ID NO:583, SEQ ID NO:584, SEQ ID NO:585, SEQ ID NO:586, SEQ ID NO:587, SEQ ID NO:588, SEQ ID NO:589, SEQ ID NO:590, SEQ ID NO:591, SEQ ID NO:592, SEQ ID NO:593, SEQ ID NO:594, SEQ ID NO:595, SEQ ID NO:596, SEQ ID NO:597, SEQ ID NO:598, SEQ ID NO:599, SEQ ID NO:600, SEQ ID NO:601, SEQ ID NO:602, SEQ ID NO:603, SEQ ID NO:604, SEQ ID NO:605, SEQ ID NO:606, SEQ ID NO:607, SEQ ID NO:608, SEQ ID NO:609, SEQ ID NO:610, SEQ ID NO:611, SEQ ID NO:612, SEQ ID NO:613, SEQ ID NO:614, SEQ ID NO:615, SEQ ID NO:616, SEQ ID NO:617, SEQ ID NO:618, SEQ ID NO:619, SEQ ID NO:620, SEQ ID NO:621, SEQ ID NO:622, SEQ ID NO:623, SEQ ID NO:624, SEQ ID NO:625, SEQ ID

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17. The method of claim 15 wherein said oligonucleotide comprises DNA, RNA, cDNA, PNA, genomic DNA, or synthetic oligonucleotides.

18. The method of claim 1 wherein said expression level is detected by measuring one or more proteins expressed by said one or more genes.

19. The method of claim 18 wherein said one or more proteins comprise an amino acid sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:165, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID

NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:216, SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:240, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, SEQ ID NO:257, SEQ ID NO:258, SEQ ID NO:259, SEQ ID NO:260, SEQ ID NO:261, SEQ ID NO:262, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:265, SEQ ID NO:266, SEQ ID NO:267, SEQ ID NO:268, SEQ ID NO:269, SEQ ID NO:270, SEQ ID NO:271, SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, SEQ ID NO:279, SEQ ID NO:280, SEQ ID NO:281, SEQ ID NO:282, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:285, SEQ ID NO:286, SEQ ID NO:287, SEQ ID NO:288, SEQ ID NO:289, SEQ ID NO:290, SEQ ID NO:291, SEQ ID NO:292, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:295, SEQ ID NO:296, SEQ ID NO:297, SEQ ID NO:298, SEQ ID NO:299, SEQ ID NO:300, SEQ ID NO:301, SEQ ID NO:302, SEQ ID NO:303, SEQ ID NO:304, SEQ ID NO:305, SEQ ID NO:306, SEQ ID NO:307, SEQ ID NO:308, SEQ ID NO:311, SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, SEQ ID NO:319, SEQ ID NO:320, SEQ ID NO:321, SEQ ID NO:322, SEQ ID NO:323, SEQ ID NO:324, SEQ ID NO:325, SEQ ID NO:326, SEQ ID NO:327, SEQ ID NO:328, SEQ ID NO:329, SEQ ID NO:330, SEQ ID NO:331, SEQ ID NO:332, SEQ ID NO:333, SEQ ID NO:334, SEQ ID NO:335, SEQ ID NO:336, SEQ ID NO:337, SEQ ID NO:338, SEQ ID NO:339, SEQ ID NO:340, SEQ ID NO:341, SEQ ID NO:342, SEQ ID NO:343, SEQ ID NO:344, SEQ ID NO:345, SEQ ID NO:346, SEQ ID NO:347, SEQ ID NO:348, SEQ ID NO:349, SEQ ID NO:350, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:354, SEQ ID NO:355, SEQ ID NO:356, SEQ ID NO:357, SEQ ID NO:358, SEQ ID NO:359, SEQ ID NO:360, SEQ ID NO:361, SEQ ID NO:362, SEQ ID NO:363, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:367, SEQ ID NO:368, SEQ ID NO:369, SEQ ID NO:370, SEQ ID NO:371, SEQ ID NO:372, SEQ ID NO:373, SEQ ID NO:374, SEQ ID NO:375, SEQ ID NO:376, SEQ ID NO:377, SEQ ID NO:378, SEQ ID NO:379, SEQ ID NO:380, SEQ ID NO:381, SEQ ID NO:382, SEQ ID NO:383, SEQ ID NO:384, SEQ ID NO:385, SEQ ID NO:386, SEQ ID NO:387, SEQ ID NO:388, SEQ ID NO:389, SEQ ID NO:390, SEQ ID NO:391, SEQ ID NO:392, SEQ ID NO:393, SEQ ID NO:394, SEQ ID NO:395, SEQ ID NO:396, SEQ ID NO:397, SEQ ID NO:398, SEQ ID NO:399, SEQ ID NO:400, SEQ ID NO:401, SEQ ID NO:402, SEQ ID NO:403, SEQ ID NO:404, SEQ ID NO:405, SEQ ID NO:406, SEQ ID NO:407, SEQ ID NO:408, SEQ ID

NO:409, SEQ ID NO:411, SEQ ID NO:412, SEQ ID NO:413, SEQ ID NO:414, SEQ ID NO:415, SEQ ID NO:416, SEQ ID NO:417, SEQ ID NO:418, SEQ ID NO:419, SEQ ID NO:421, SEQ ID NO:422, SEQ ID NO:423, SEQ ID NO:424, SEQ ID NO:425, SEQ ID NO:426, SEQ ID NO:427, SEQ ID NO:428, SEQ ID NO:429, SEQ ID NO:430, SEQ ID NO:431, SEQ ID NO:432, SEQ ID NO:433, SEQ ID NO:434, SEQ ID NO:435, SEQ ID NO:436, SEQ ID NO:437, SEQ ID NO:438, SEQ ID NO:439, SEQ ID NO:440, SEQ ID NO:441, SEQ ID NO:442, SEQ ID NO:443, SEQ ID NO:444, SEQ ID NO:445, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:449, SEQ ID NO:450, SEQ ID NO:451, SEQ ID NO:452, SEQ ID NO:453, SEQ ID NO:454, SEQ ID NO:455, SEQ ID NO:456, SEQ ID NO:457, SEQ ID NO:458, SEQ ID NO:459, SEQ ID NO:460, SEQ ID NO:461, SEQ ID NO:462, SEQ ID NO:463, SEQ ID NO:464, SEQ ID NO:465, SEQ ID NO:466, SEQ ID NO:467, SEQ ID NO:468, SEQ ID NO:470, SEQ ID NO:471, SEQ ID NO:472, SEQ ID NO:473, SEQ ID NO:474, SEQ ID NO:475, SEQ ID NO:476, SEQ ID NO:477, SEQ ID NO:478, SEQ ID NO:479, SEQ ID NO:480, SEQ ID NO:481, SEQ ID NO:482, SEQ ID NO:483, SEQ ID NO:484, SEQ ID NO:485, SEQ ID NO:486, SEQ ID NO:487, SEQ ID NO:488, SEQ ID NO:489, SEQ ID NO:490, SEQ ID NO:491, SEQ ID NO:492, SEQ ID NO:493, SEQ ID NO:494, SEQ ID NO:495, SEQ ID NO:496, SEQ ID NO:497, SEQ ID NO:498, SEQ ID NO:499, SEQ ID NO:500, SEQ ID NO:501, SEQ ID NO:502.

20. The method of claim 2 wherein said expression level of said one or more genes is detected by measuring one or more proteins expressed by said one or more genes, and said expression level of said one or more additional genes is detected by measuring one or more proteins expressed by said one or more additional genes.

21. The method of claim 20, wherein said one or more proteins expressed by said one or more genes comprise an amino acid sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID

NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:165, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:216, SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:240, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, SEQ ID NO:257, SEQ ID NO:258, SEQ ID NO:259, SEQ ID NO:260, SEQ ID NO:261, SEQ ID NO:262, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:265, SEQ ID NO:266, SEQ ID NO:267, SEQ ID NO:268, SEQ ID NO:269, SEQ ID NO:270, SEQ ID NO:271, SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, SEQ ID NO:279, SEQ ID NO:280, SEQ ID NO:281, SEQ ID NO:282, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:285, SEQ ID NO:286, SEQ ID NO:287, SEQ ID NO:288, SEQ ID NO:289, SEQ ID NO:290, SEQ ID NO:291, SEQ ID NO:292, SEQ ID

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NO:496, SEQ ID NO:497, SEQ ID NO:498, SEQ ID NO:499, SEQ ID NO:500, SEQ ID NO:501, SEQ ID NO:502, and

said one ore more proteins expressed by said one or more additional genes comprise an amino acid sequence encoded by a nucleotide sequence selected from the group consisting SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:101, SEQ ID NO:238, SEQ ID NO:309, SEQ ID NO:310, SEQ ID NO:352, SEQ ID NO:365, SEQ ID NO:410, SEQ ID NO:420, SEQ ID NO:448, SEQ ID NO:469.

22. The method of claim 18, wherein said measuring comprises measuring serum.

23. The method of claim 18, wherein said protein is a cell surface protein.

24. The method of claim 18, wherein said measuring comprises using a fluorescent activated cell sorter

25. The method of claim 1, wherein the expression level detected is expression level in the patient's bodily fluid.

26. The method of claim 25, wherein said bodily fluid is peripheral blood.

27. The method of claim 1, further comprising selecting an appropriate therapy.

28. The method of claim 27 wherein said therapy includes administration of a drug that targets alpha-interferon.

29. A system for detecting gene expression in body fluid comprising at least two isolated polynucleotides wherein the isolated polynucleotides detect expression of a gene wherein the gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:503, SEQ ID NO:505, SEQ ID NO:506, SEQ ID NO:508, SEQ ID NO:509, SEQ ID NO:510, SEQ ID NO:511, SEQ ID NO:512, SEQ ID NO:513, SEQ ID NO:514, SEQ ID NO:515, SEQ ID NO:516, SEQ ID NO:517, SEQ ID NO:518, SEQ ID NO:519, SEQ ID NO:520, SEQ ID NO:521, SEQ ID NO:522, SEQ ID NO:523, SEQ ID NO:524, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:540, SEQ ID NO:541, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, SEQ ID NO:545, SEQ ID NO:546, SEQ ID NO:547, SEQ ID NO:548, SEQ ID NO:549, SEQ ID NO:550, SEQ ID NO:551, SEQ ID NO:552, SEQ ID NO:553, SEQ ID NO:554, SEQ ID NO:555, SEQ ID NO:556, SEQ ID NO:557, SEQ ID NO:558, SEQ ID NO:559, SEQ ID NO:560, SEQ ID NO:561, SEQ ID NO:562, SEQ ID NO:563, SEQ ID NO:564, SEQ ID NO:565, SEQ ID NO:566, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:569, SEQ ID NO:570, SEQ ID NO:571, SEQ ID NO:572, SEQ ID NO:573, SEQ ID NO:574, SEQ ID NO:575, SEQ ID NO:576, SEQ ID NO:577, SEQ ID NO:578, SEQ ID NO:579, SEQ ID NO:580, SEQ ID NO:581, SEQ ID NO:582, SEQ ID NO:583, SEQ ID NO:584, SEQ ID NO:585, SEQ ID NO:586, SEQ ID NO:587, SEQ ID NO:588, SEQ ID NO:589, SEQ ID NO:590, SEQ ID NO:591, SEQ ID NO:592, SEQ ID NO:593, SEQ ID NO:594, SEQ ID NO:595, SEQ ID NO:596, SEQ ID NO:597, SEQ ID NO:598, SEQ ID NO:599, SEQ ID NO:600, SEQ ID NO:601, SEQ ID NO:602, SEQ ID NO:604, SEQ ID NO:605, SEQ ID NO:606, SEQ ID

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inflammatory disease related symptom compared to the expression of the gene in leukocytes in an individual not experiencing said symptom.

Figure 1: Novel Gene Sequence Analysis

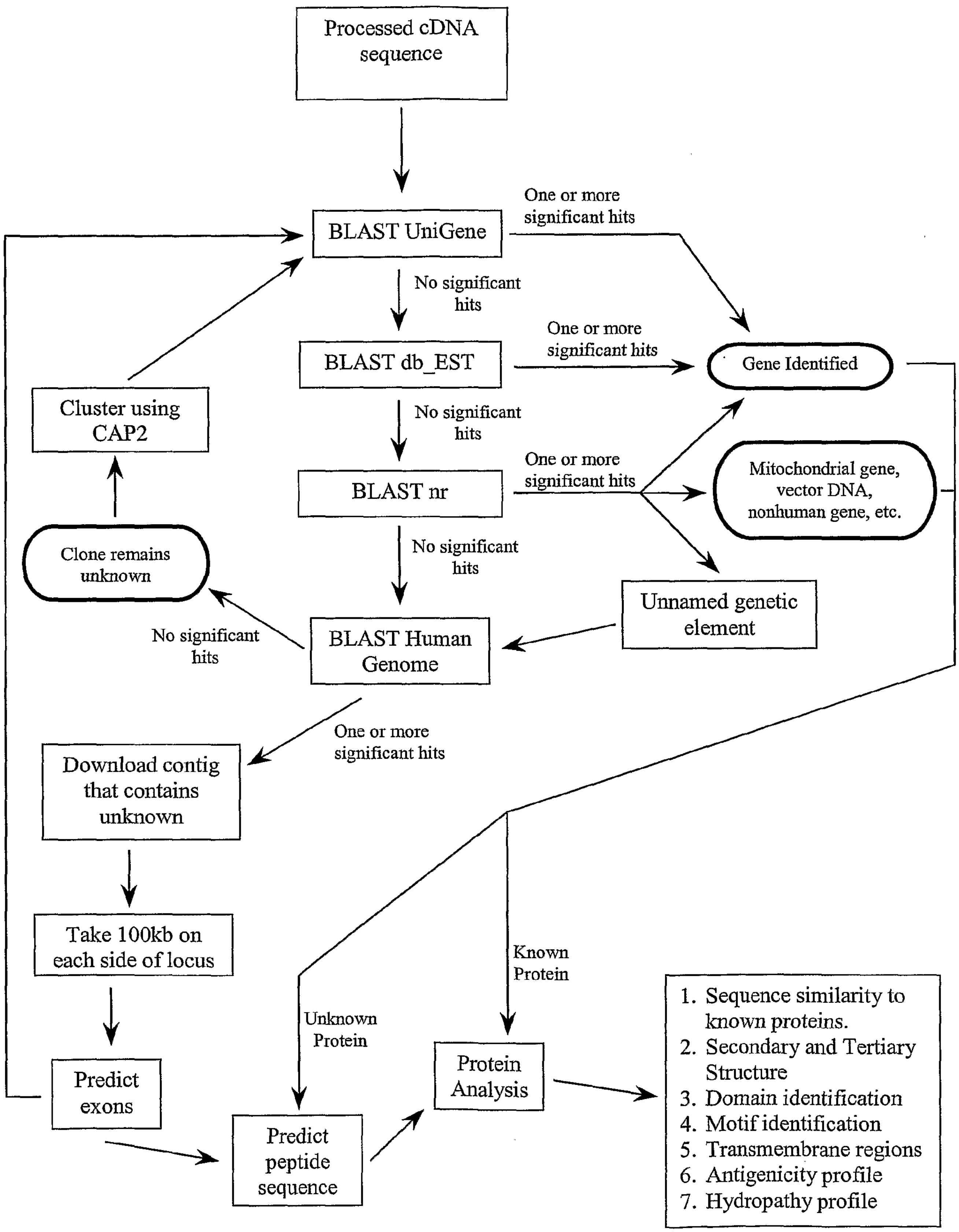


Figure 2: Primer efficiency testing. A standard curve of Ct versus log of the starting RNA amount is shown for 2 genes.

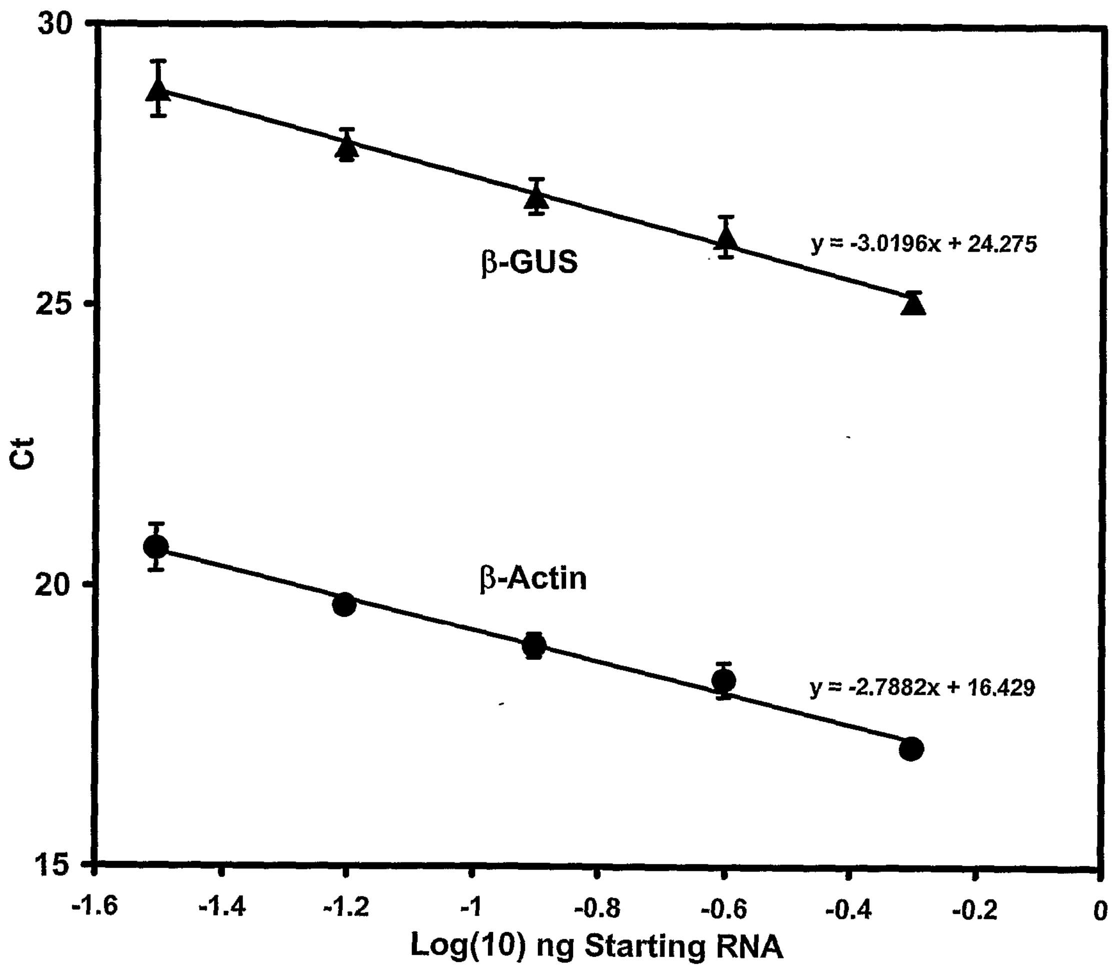


Figure 3: Kits for discovery of, or application of diagnostic gene sets

A. Contents of kit for discovery of diagnostic gene sets using microarrays

1. Sterile, endotoxin and RNAse free blood collection tubes
2. Alcohol swabs, tourniquet, blood collection set
3.-PBS (phosphate buffer saline; needed when method of example 8 is used to derived mononuclear RNA)
4. Cell lysis buffer
5. RNA isolation kit
6. Substrates for labeling of RNA (may vary for various expression profiling techniques)
   For fluorescence microarray expression profiling:
   Reverse transcriptase and 10x RT buffer
   T7(dT)24 primer (primer with T7 promoter at 5' end)
   DTT
   Deoxynucleotides 100mM each
   RNAse inhibitor
   2nd strand cDNA buffer
   DNA polymerase
   Rnase H
   T7 RNA polymerase
   Ribonucleotides
   In Vitro transcription buffer
   Cy3 and Cy5 labeled ribonucleotides
7. Microarrays containing candidate gene libraries
8. Cover slips for slides
9. Hybridization chambers
10. Software package for identification of diagnostic gene set from data
    Contains statistical methods.
    Allows alteration in desired sensitivity and specificity of gene set.
    Software facilitates access to and data analysis by centrally located database server.
11. Password and account number to access central database server.
12. Kit User Manual

B. Contents of kit for application of diagnostic gene sets using microarrays

1. Sterile, endotoxin and RNAse free blood collection tubes
2. Alcohol swabs, tourniquet, blood collection set
3.-PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA)
4. Cell lysis buffer
5. RNA isolation kit
6. Substrates for labeling of RNA (may vary for various expression profiling techniques)
   For fluorescence microarray expression profiling:

Reverse transcriptase and 10x RT buffer
T7(dT)24 primer (primer with T7 promoter at 5' end)
DTT
Deoxynucleotides 100mM each
RNAse inhibitor
2nd strand cDNA buffer
DNA polymerase
Rnase H
T7 RNA polymerase
Ribonucleotides
In Vitro transcription buffer
Cy3 and Cy5 labeled ribonucleotides

7. Microarrays containing candidate gene libraries
8. Cover slips for slides
9. Hybridization chambers
10. Software package for identification of diagnostic gene set from data
    Contains statistical methods.
    Allows alteration in desired sensitivity and specificity of gene set.
    Software facilitates access to and data analysis by centrally located database server.
11. Password and account number to access central database server.
12. Kit User Manual

C. Contents of kit for application of diagnostic gene sets using Real-time RT-PCR

1. Sterile, endotoxin and RNAse free blood collection tubes
2. Alcohol swabs, tourniquet, blood collection set
3.-PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA)
4. Cell lysis buffer
5. RNA isolation kit
6. Substrates for real time RT-PCR (may vary for various real-time PCR techniques:
    poly dT primers, random hexamer primers
    Reverse Transcriptase and RT buffer
    DTT
    Deoxynucleotides 100 mM
    RNase H
    primer pairs for diagnostic and control gene set
    10x PCR reaction buffer
    Taq DNA polymerase
    Fluorescent probes for diagnostic and control gene set
    (alternatively, fluorescent dye that binds to only double stranded DNA)
    reaction tubes with or without barcode for sample tracking
    96-well plates with barcode for sample identification, one barcode for entire set, or individual barcode per reaction tube in plate
7. Software package for identification of diagnostic gene set from data
    Contains statistical methods.
    Allows alteration in desired sensitivity and specificity of gene set.

Software facilitates access to and data analysis by centrally located database server

8. Password and account number to access central database server.
9. Kit User Manual

FIGURE 4

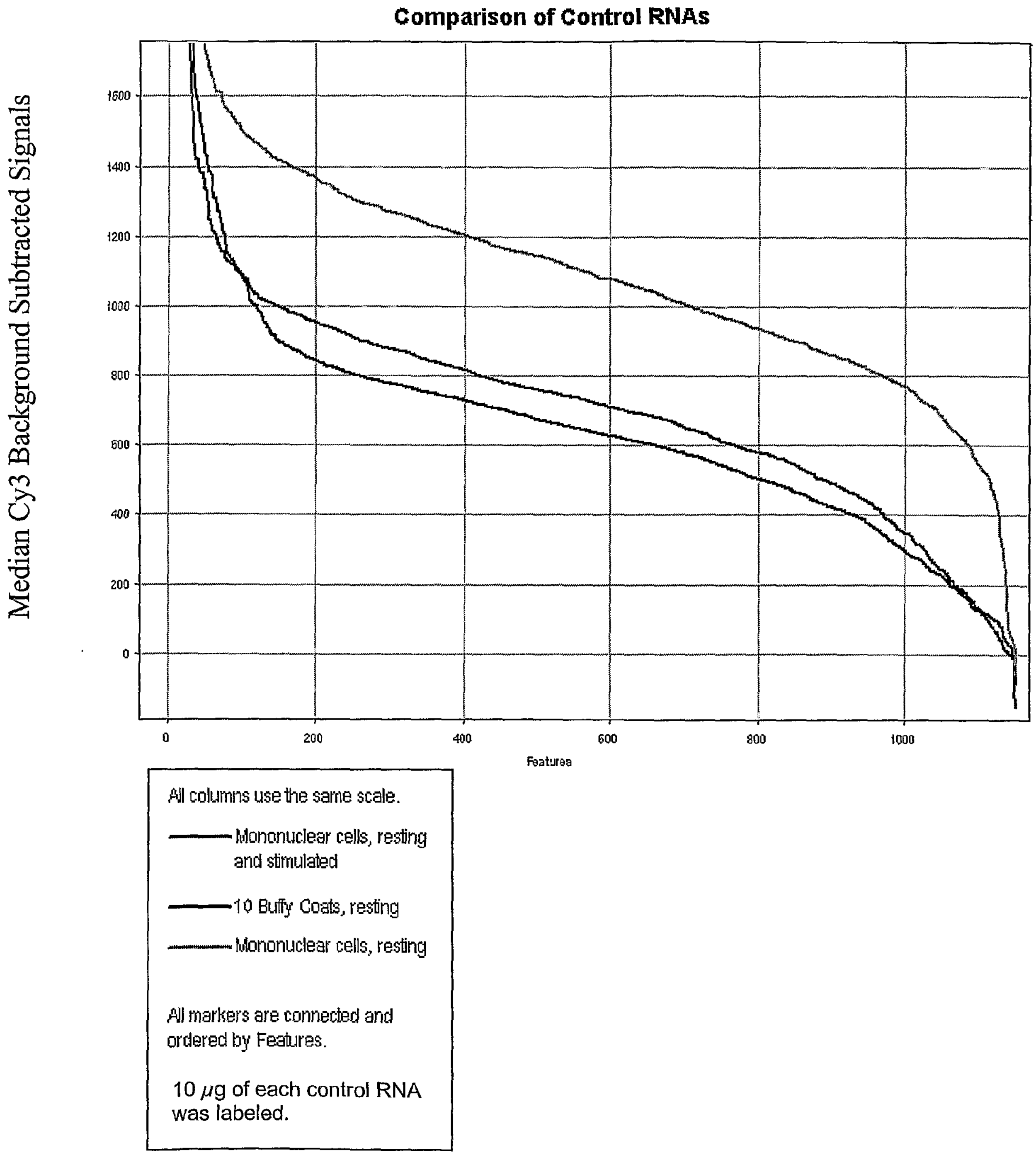
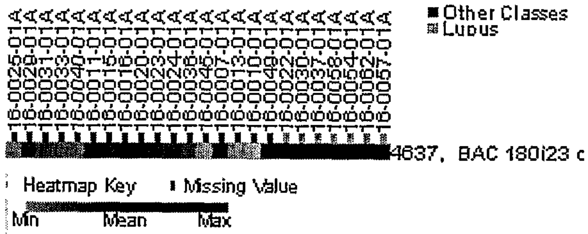

Comparison of Control RNAs
Median Cy3 Background Subtracted Signals
1600
1400
1200
1000
800
600
400
200
0
0
200
400
600
800
1000
Features
All columns use the same scale.
Mononuclear cells, resting and stimulated
10 Buffy Coats, resting
Mononuclear cells, resting
All markers are connected and ordered by Features.
10 μg of each control RNA was labeled.

Figure 5: SLE diagnostic genes and algorithms

A.



FDR: 0.5

# of significant genes: 1

# of false significant genes: 0.0050

B.

Lupus		Control	
Sample	Ratio	Sample	Ratio
16-0022-01	1.05	16-0025-01	0.60
16-0030-01	0.96	16-0029-01	0.75
16-0037-01	0.87	16-0031-01	0.63
16-0058-01	1.05	16-0033-01	0.62
16-0054-01	0.99	16-0040-01	0.61
16-0062-01	0.98	16-0015-01	0.72
16-0057-01	1.14	16-0016-01	0.78
		16-0020-01	0.79
		16-0023-01	0.71
		16-0024-01	0.69
		16-0036-01	0.65
		16-0045-01	0.59
		16-0007-01	0.77
		16-0013-01	0.60
		16-0010-01	0.57
		16-0049-01	0.75

	Lupus	Controls
Average Ratio	1.00	0.68
Std Dev of Ratio	0.08	0.08
Fold Change	1.48	

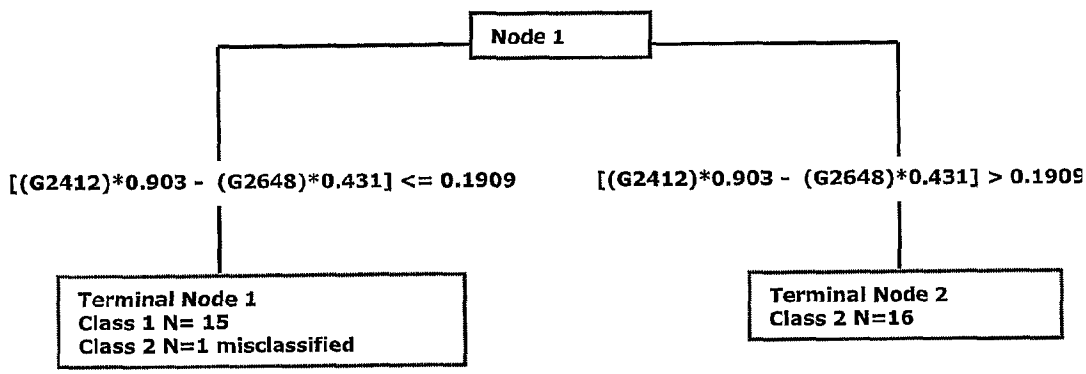
C.

Model	# genes	Relative Cost	SEQ ID 50mer	Locus	Nominal Description	CART Splitter	CART Value for Dx SLE
Model I	2	0.118	514	NM_002946	replication protein A2 (32kD)	co-1st	[(2412)*0.903 - (2648)*0.431] <= 0.1909
			510	NM_004510	interferon-induced protein 75	co-1st	[(2412)*0.903 - (2648)*0.431] <= 0.1909
Model I	3	0.125	514	NM_002946	replication protein A2 (32kD)	co-1st	[(2412)*0.903 - (2648)*0.431] <= 0.1909
			510	NM_004510	interferon-induced protein 75	co-1st	[(2412)*0.903 - (2648)*0.431] <= 0.1909
			509	BC002409	actin, beta (ACTB)	2nd	(G1436) > 0.0868
Model II	1	0.612	504	W16552	PKR	1st	(5067) > 0.1030
Model II	3	0.686	504	W16552	PKR	1st	(5067) > 0.1030
			875	AK024756	hypothetical protein FLJ21103	2nd	(G1025) <= 0.3968
			876	AK024969	hypothetical protein DKFZp566I133	3rd	(G1035) <= 0.0073
Model II	5	0.745	504	W16552	PKR	1st	(5067) > 0.1030
			874	AK024240	cDNA FLJ14178 fis	2nd	(G1003) > 0.2105
			875	AK024756	hypothetical protein FLJ21103	2nd	(G1025) <= 0.3968
			873	AK024202	heat shock 90kD protein 1, alpha	3rd	(G1001) <= - 0.3107
			876	AK024969	hypothetical protein DKFZp566I133	3rd	(G1035) <= 0.0073

D.

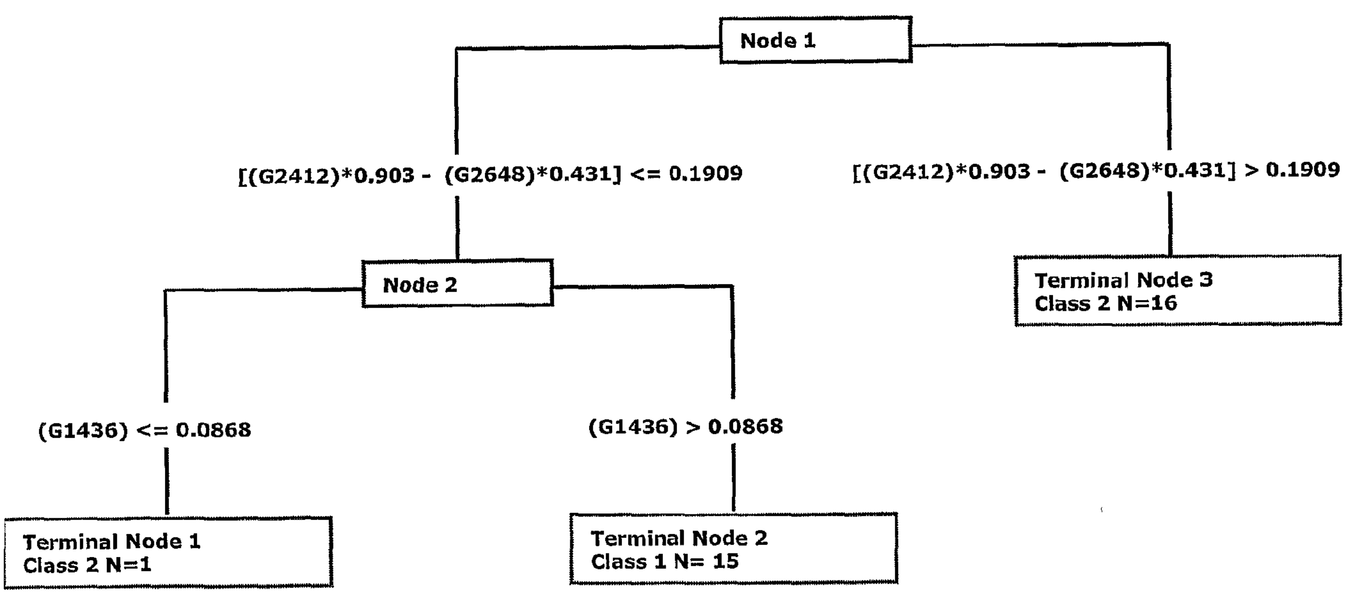
	Model	Sensitivity	Specificity	Relative Cost
Training Set	Model 1 (2 genes)	100	94	
	Model 1 (3 genes)	100	100	
10-fold Cross Validation	Model 1 (2 genes)	100	88	0.118
	Model 1 (3 genes)	93	94	0.125

E.



Model I (2 genes)

F.



Model 1 (3 genes)

Figure 6. Endpoint testing of PCR primers

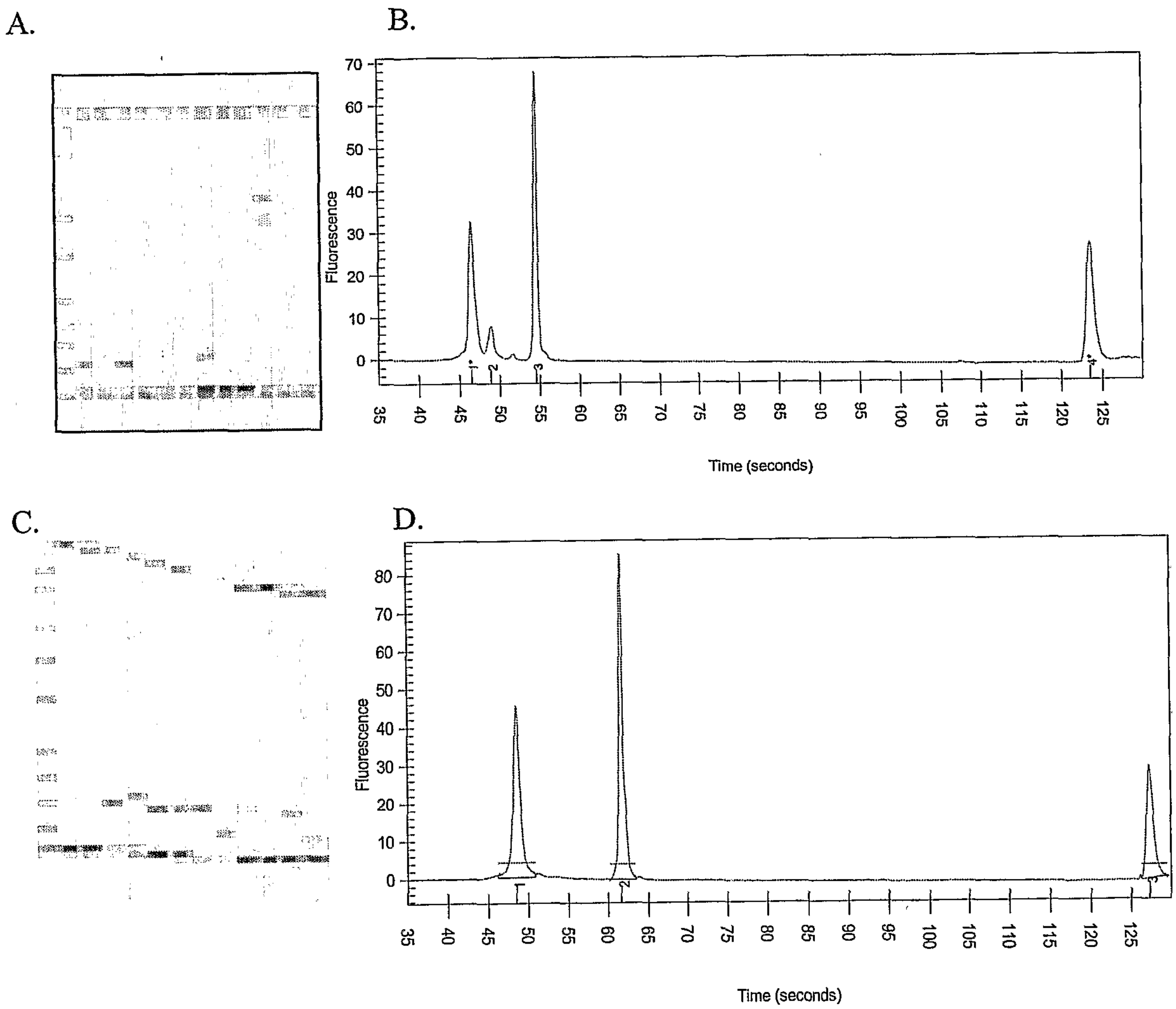
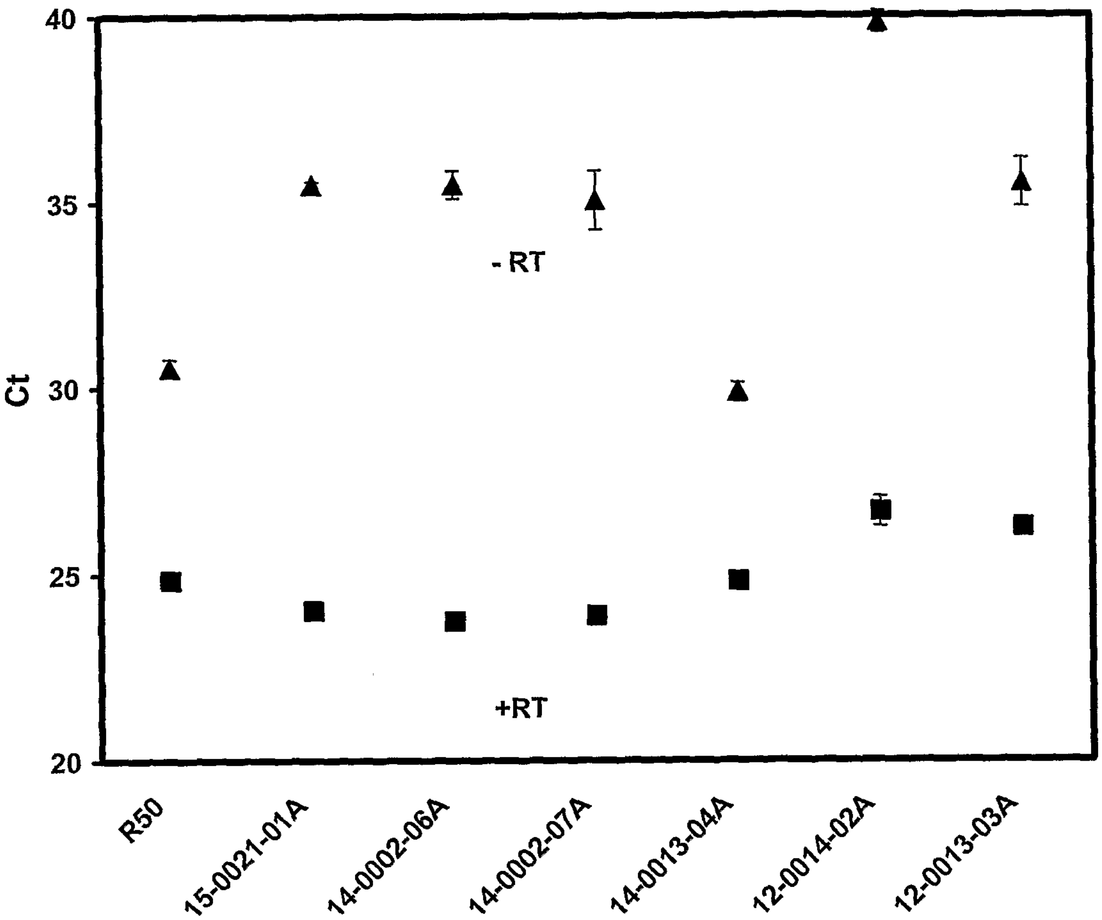


Figure 7: Validation of differential expression of Granzyme B in CMV patients using Real-time PCR

A.



B.

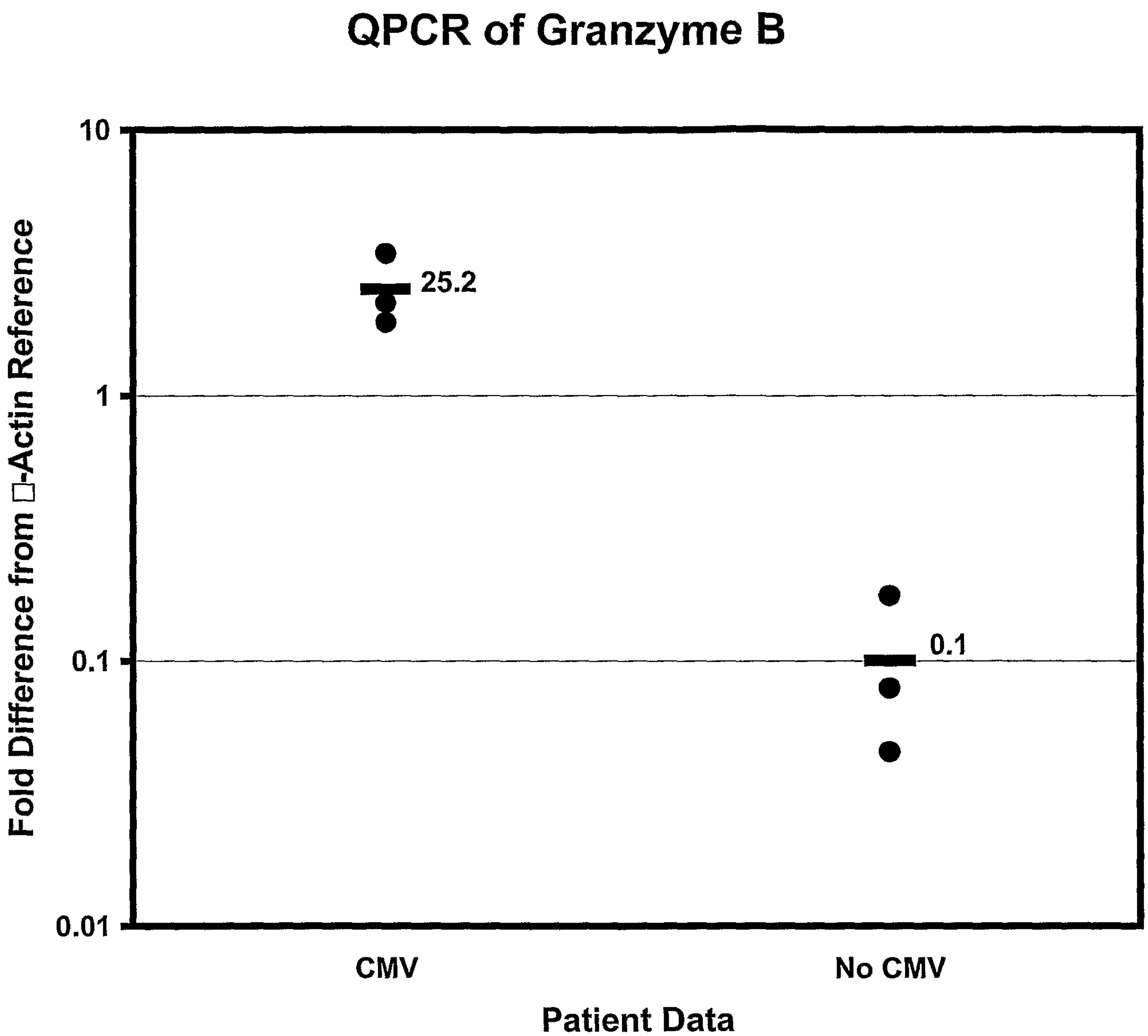
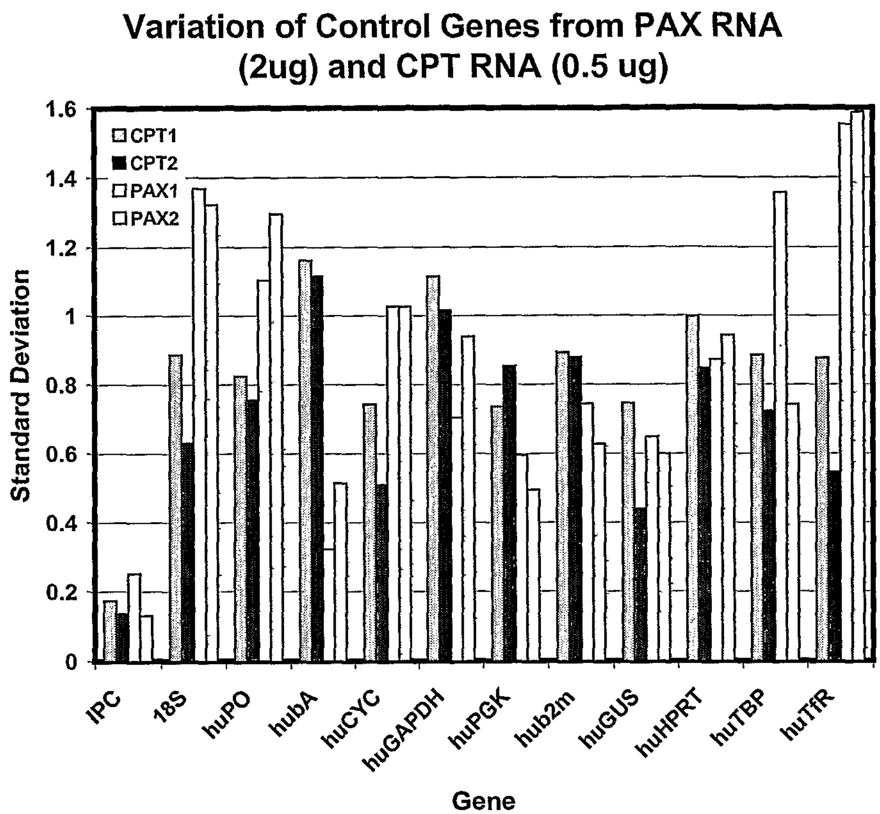
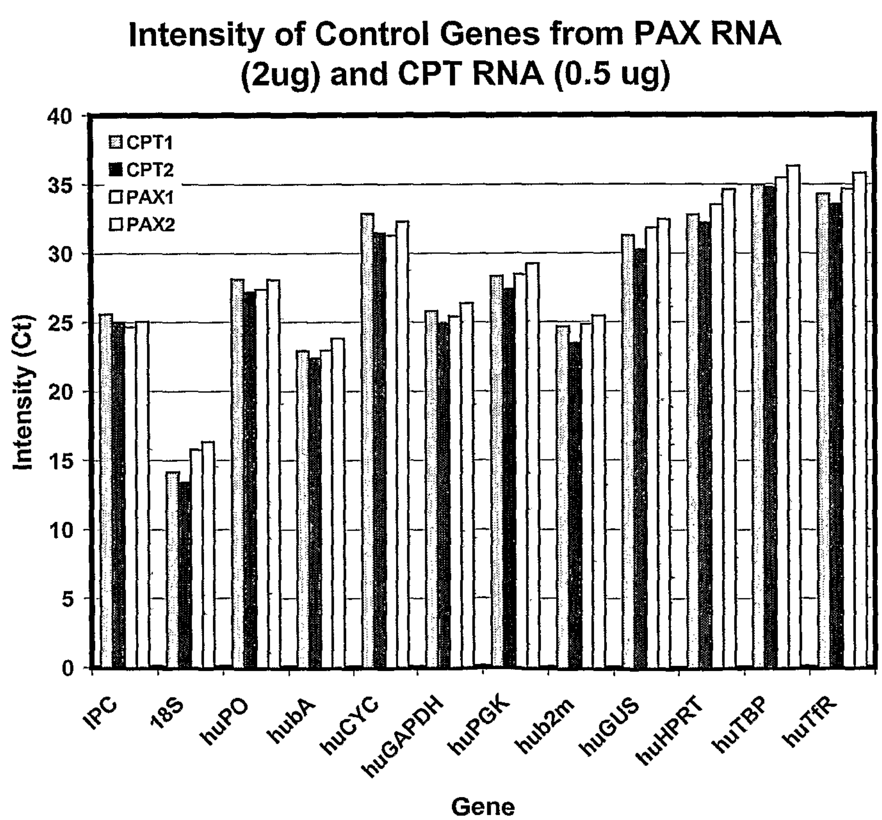

QPCR of Granzyme B
10
1
0.1
0.01
Fold Difference from □-Actin Reference
25.2
0.1
CMV
No CMV
Patient Data

Figure 8


Variation of Control Genes from PAX RNA (2ug) and CPT RNA (0.5 ug)
CPT1
CPT2
PAX1
PAX2
Standard Deviation
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IPC
18S
huPO
hubA
huCYC
huGAPDH
huPGK
hub2m
huGUS
huHPRT
huTBP
huTfR
Gene


Intensity of Control Genes from PAX RNA (2ug) and CPT RNA (0.5 ug)
CPT1
CPT2
PAX1
PAX2
Intensity (Ct)
40
35
30
25
20
15
10
5
0
IPC
18S
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huCYC
huGAPDH
huPGK
hub2m
huGUS
huHPRT
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huTfR
Gene

SEQUENCE LISTING

<110> EXPRESSION DIAGNOSTICS, INC.
Wohlgemuth, Jay
Fry, Kirk
Woodward, Robert
Ly, Ngoc

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tcagaaccca tgcggcagca aggcctgccg ccgcctcttc ggcccagtgg acagcgagca 180

gctgagccgc gactgtgatg cgctaatggc gggctgcatc caggaggccc gtgagcgatg 240

gaacttcgac tttgtcaccg agacaccact ggagggtgac ttcgcctggg agcgtgtgcg 300

gggccttggc ctgcccaagc tctaccttcc cacggggccc cggcgaggcc gggatgagtt 360

gggaggaggc aggcggcctg gcacctcacc tgctctgctg caggggacag cagaggaaga 420

ccatgtggac ctgtcactgt cttgtaccct tgtgcctcgc tcaggggagc aggctgaagg 480

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tttctaccac tccaaacgcc ggctgatctt ctccaagagg aagccctaat ccgcccacag 600

gaagcctgca gtcctggaag cgcgagggcc tcaaaggccc gctctacatc ttctgcctta 660

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ggcggttgaa tgagaggttc ctaagagtgc tgggcatttt tattttatga aatactattt 840

aaagcctcct catcccgtgt tctccttttc ctctctcccg gaggttgggt gggccggctt 900

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<211> 556
<212> DNA
<213> Homo sapiens

<400> 132
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gttttccagt ttcttaatgc gaaatgcgag tcggctttcc tttccaagag gaatcctcgg 180

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<213> Homo sapiens

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cgtaatgagg atgaagattc accaaataag ctatatactt tggttaccta tgtacctgtt 360

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<211> 402
<212> DNA
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tggtttttttc attttggttt tgccacaaaa agagcaaggg tacttggggg gctggctgat 300

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aaacccgact tttttgggac gtttgggcat gtcgccgcaa cc 402

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<211> 2266
<212> DNA
<213> Homo sapiens

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<213> Homo sapiens

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aaaggcttta ggatataaat ttcatgttac agagcatgtc attgtcaaag gaaatctgtg 360

gccctgagat tttaagaaca taaaatgtga catttgatat ttctccagcc cagggaagta 420

agatggttag caatggttgc cttaatcaaa tggtcccatt tttaacccca aaggaagtgc 480

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tgaaaggccc cttaggtcag atcctgagag tagcacattt gagtgcagat tcctgggccc 600

cacctcaaac ctactaattc tgaatctctg ggaatagggc caggaaatct gccctttcta 660

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<211> 1865
<212> DNA
<213> Homo sapiens

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<211> 1198
<212> DNA
<213> Homo sapiens

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<211> 453
<212> DNA
<213> Homo sapiens

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<223> n is a, c, g, t or u

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<210> 141
<211> 222
<212> DNA
<213> Homo sapiens

<220>
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<223> n is a, c, g, t or u

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gttcaccatc acaactgcat taccaaaagg ggatttttnc aaacgcggag ttgaccaaaa 180

taatatctga ggatgattgc ttttccctgc tgccagctga tc 222

<210> 142
<211> 1851
<212> DNA

<213> Homo sapiens

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gatgggctgt ctgacatttt g 81

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<213> Homo sapiens

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ggatgcaagc cttgccttcc agaagcttac cagtcgggtt gccagcaaag cagtggatgc 660

aagacttgcc ctccaggagc ttaccatcac aacgaagaag acaaataaat gcataatata 720

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ttcaccatgt tggtcaggct ggtctcgatc tcttgacctc gcgatccact cgcctcagcc 240

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cctctgtgtc tggcgtgtct cattgagcct ggagtgtctc attgagcctg gcgtcctgaa 180

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agtgcatgga gggccacact acgcctcctc ctctgctgac ggccatctgg gttgtagcca 300

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ttccactc 368

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<211> 239
<212> DNA
<213> Homo sapiens

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tgaacaccct gagagtcgag gacacggcta tatattattg cgcgagagac cgagggaaat 180

tatattgtag tggtggtatt tgctttccgc ctgttggcta cttcgacccc tggggccaa 239

<210> 279
<211> 335
<212> DNA
<213> Homo sapiens

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catatttgat atatcaagtg tcttcacaac tgtgcttgca tattctttcc caaaatattg 180

aaagtccata tatttccttg tacattttta aagttgatat ctaaatcttt catgtagttg 240

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<223> n is a, c, g, t or u

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gatttgaaag aggcaacctt tttcctcgcg tttctaaagg cctatgaaaa gggcacgtcg 240

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<212> DNA
<213> Homo sapiens

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<211> 3624
<212> DNA
<213> Homo sapiens

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<211> 456
<212> DNA
<213> Homo sapiens

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taaaccaacc gccacagagt agatgttcaa tcaaagtgag ctgttaatga caaggttatt 240

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<211> 406
<212> DNA
<213> Homo sapiens

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gccccttccc aaccattcca ccaccaccca aaagggccta gggtgggggg cttgcactgt 360

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<211> 473
<212> DNA
<213> Homo sapiens

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<222> (433)..(433)
<223> n is a, c, g, t or u

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taggtagaaa gacactctnt ctatgttcct accacttctg agtggacctg aataaacaga 420

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<211> 500
<212> DNA
<213> Homo sapiens

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cgcacagggc tagtttccag cagcggcggc gccccctttcc ctgccccacc acgcgacgtc 180

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<212> DNA
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<211> 474
<212> DNA
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ccactaatac acactttgaa aagtaccatc accatatata tatttgcttt aaaaaattat 180

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ccccggccct ttaaaaggga aaaaaacaag gacttttttta aacccgaaaa ccccctttttt 360

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<210> 389
<211> 462
<212> DNA
<213> Homo sapiens

<400> 389

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acaaggagct aataccccaa tatatacaga gctcagaagt tattatgaaa gacattaaca     180

tatagcaaaa caagcaatgg ccatgtggta tcacagaaaa ttctggaatt tcatatcaag     240

ggtgatagga ggctcttttg ttttagtgag acaatttttt tttttttttt tgagacacag     300

tctcgctctg tcacccaggc tggagtgaag tggtgcgatc tcggctcact gcaagctccg     360

cctcccaggt tcacgccatt ctcctgcctc agcctcccga gtagctggga ctacaggtgc     420

ccgccaccaa gcctggctaa tttttgtat ttttagtaga ga                         462
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<213>  Homo sapiens

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tagctaattt tgtaggggac ctgattttta gtccagcttg gctggcaact aattttaggt     120

ctgtaaaggt tcagaaagca tatcctgaac acaagccctc ctcagttacg ttatttaaag     180

tgttaaatac tcaagccaac cgaaacacaa accaaagtaa agaatttaga taagaaagac     240

atgtgaaaag gaggctactg gtaagtacag aactcagtta aatgtaaata attatgaatt     300

aattgtatta tctttttatt taaaaatcta ataaattctg atttttctct ccccaacttc     360

ctgtgatata actaagaaaa aacaaagaga aactagtttc tgtaaaactg gaaactccga     420

gaattcctca gtgatatgcc aggaaacagg aagaatttcc actagccaaa gttctgagga     480

agttacaggc aggaaaaaag ataagggtta ccatcttttt ttagtcaata aagctatgcc     540

cactctaggt actttcctta gaaacatgga gtcttcccag cagagaaagg aaagctag       598
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<211>  383
<212>  DNA
<213>  Homo sapiens


<220>
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<222>  (341)..(341)
<223>  n is a, c, g, t or u

<220>
<221>  misc_feature
<222>  (346)..(346)
<223>  n is a, c, g, t or u

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<221> misc_feature
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<223> n is a, c, g, t or u

<400> 391
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atattgaaaa cagcctgtaa gttttcatcc aatatttaaa accacctcct ggaactaaaa 180

ttggtcttca aaaatcatgg gcgtattaac attttccaaa catgccctgc tggactagga 240

aggtcctgtt attctttctt ttgaacttcc cagtaagttt ccttgttccc tattcctagg 300

gtttaaagtg gcaaagggac tttttatgag gctattaggg ncaagntttc ttccattgga 360

aaatnaaact tttggcggga aat 383

<210> 392
<211> 573
<212> DNA
<213> Homo sapiens

<220>
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<223> n is a, c, g, t or u

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agggcacaca gttccaacac cagaacagag aatttgggaa gataattgct ctgaaacaga 180

actggcctcc ctgtgtctat tagaaaacat ttccaaagct cacggaggga ggccaacttc 240

ccctatggga aacccattca ctcgccaaag ggcagaaggc atcataaatc acccattgat 300

acattggtgg ggggctcctg tccccctggt gaccactcca aggtgatttg atctgtgctt 360

cctctgttgg gtcagagacg aaacgggcta ttattaggtc aaacattaca gaaatcaact 420

gagactctta actagtagtt gatacaccac agggctttac tttactgcac aattactaac 480

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aaagcatgac agggtttgaa cagtgatctt gaa 573

<210> 393
<211> 497
<212> DNA
<213> Homo sapiens

<400> 393
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ttcccagaca gcccagcctg ctccccttag gaatgagtgt ccctggaggg ggagagcctg 180

gaaccaaagc cccgccagga actgcttccc ctaaactgag gttctctgaa aaaaatgttc 240

gcctggctga taaagccgcc tcttaacaga gcccagacac ttctgtgctt cccctgggtt 300

gctaattgag gacactaaag ccctaagaga taccccaggt cgggggaagg ggccccaaga 360

cctagacctc cggtggcgac catgcccttg agaggatggg agctgaattg gagcacgaga 420

ttatttatca tcgctggatg aagctccagc tagagctcag tatttcctct ttttctgggc 480

tcagacagac acagact 497

<210> 394
<211> 505
<212> DNA
<213> Homo sapiens

<400> 394
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ggatgctcag aaaacgtaag aagctgaagg gaaaacacat catctgtgta ctcagacaca 180

cacactccaa cccatcacac gaacacaccc tcgcccgccc atcagagaag aattcgcctg 240

gaatcagctg gggcggtgg ctcacgccta taatcccagc actttgggag gttgaggcgg 300

gcagatcatg aggtcaggag ttcacgacca gcctgaccaa catggtgaaa ccctatctct 360

actaaaaata caaaaatcag cggggcctgg tggcatgcac ctgtaatccc agctactcag 420

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cactcctgcc tgggcaacag agcaa 505

<210> 395
<211> 2283
<212> DNA
<213> Homo sapiens

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actcagaagc tttccttgtt ttcttcaggc ctgctcccag ttttcttaac agtttctttt 180

gttgctttct ctctcccttg ttgctttcca tggcagtaat cctcctagag tccaagcagt 240

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<210> 396
<211> 1634
<212> DNA
<213> Homo sapiens

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gggtcgactc ggggacggcc tgggtgctgc cctgggcccg gggaacagaa cacacatctg 180

gctttttgtt agaggtcttc atggaaagag tggtacatgg tgggatgagc atctttctga 240

agaaaatgtc ccattcatta agcagttggt ctctgatgaa gataaagccc aattagcaag 300

taaactgtgt cctctgaaag atgaaccatg gcctatacat ccttgggaac caggttcctt 360

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aaagcatgtg gtcacattac ttcaggtaca agactgtcat gtcttaaaat atacgtcaaa 480

ggaaaactgt aatggaaaaa tggcaaccct gtctgtagga ggaaaaactg tatcacgttt 540

tcgtaaagct acatccatat tggaatttta ccgggaactt ggattgccgc cgaaacagac 600

agttaaaatc tttaatataa cagataatgc tgcaattaaa ccaggcactc ctctttatgc 660

tgctcacttt cgtccaggac agtatgtgga tgtcacagcc aaaactattg gtaaaggttt 720

tcaaggtgtc atgaaaagat ggggatttaa aggccagcct gctacgcatg gtcaaacgaa 780

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taaaatgcct ggaaaaatgg gaaacatata caggacagaa tatggactga aagtgtggag 900

aataaacaca aagcacaaca taatctatgt aaatggctct gtacctggac ataaaaattg 960

cttagtaaag gtcaaagatt ctaaactgcc tgcatataag gatctcggta aaaatctacc 1020

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ggactcgatt ctttttact acatatttcc caagttatct taagatgtct gtaaatttaa 1440

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atggggacta tagtgcaacc tatttgggta aagaaaccat ttgctaaaat ggagaaagta 1560

aatagatttt tatttaaatt acagaaacat gttaaaggcc ggacaaagga aagacaataa 1620

aatcataaat tatc 1634

<210> 397
<211> 1943
<212> DNA
<213> Homo sapiens

<400> 397
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gaaggacgcg gcgtctgggg agcacccagg cagcaagacg gggcccgggc tttcgacagt 180

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gttcagtggc ttcctgaagt gactaaacct gaaggaaaag attatgccct tcattggcta 480

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cccaatgatg atgcacagtt tgatgcttcc cattgtgaca gtgacaaggg tgaatttggt 600

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tctgatgtgt tggtttttga ctatacaaaa caccctgcta aaccagaccc aagtggagaa 780

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tcaaatttga gtggacatct cctaagtgca tctgatgacc atactgtttg tctgtgggat 900

ataaacgcag gaccaaaaga aggcaaaatt gtggatgcta aagccatctt tactggccac 960

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ctgtttattc atggaggaca cactgctaag atttcagatt ttagctggaa ccccaatgag 1440

ccttgggtca tttgctcagt gtctgaggat aacatcatgc agatatggca aatggctgaa 1500

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tatcctctga ttctagcatt tcaagtgagc tgttgcgtac tgtatcatat tgtagctatt 1740

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agggtattgc ctttgattca actgttttaa gtcctcattt tctcaaacta agtgcttgct 1860

gttcccaaat atgcaagaat aacttttaca ctttttcctt ccaacacttc ttgattggct 1920

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<210> 398
<211> 594
<212> DNA
<213> Homo sapiens

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aaggagaagc tcccgcggta ctacaagaac atcggtctgg gcttcaagac acccaaggag 180

gctattgagg gcacctacat tgacaagaaa tgccccttca ctggtaatgt gtccattcga 240

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cgagactatc tgcactacat ccgcaagtac aaccgcttcg agaagcgcca caagaacatg 360

tctgtacacc tgtcccccctg cttcagggac gtccagatcg gtgacatcgt cacagtgggc 420

gagtgccggc ctctgagcaa gacagtgcgc ttcaacgtgc tcaaggtcac caaggctgcc 480

ggcaccaaga agcagttcca gaagttctga ggctggacat cggcccgctc cccacaatga 540

aataaagtta ttttctcatt ccaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa 594

<210> 399
<211> 2141
<212> DNA
<213> Homo sapiens

<400> 399
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cctgcctggc cccgggtccc gcgccccgag accgccgtcg ctcggacgct cctgctcggc 180

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<210> 400
<211> 1102
<212> DNA
<213> Homo sapiens

<400> 400
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cctcagacct tccagatcgc ttcctctcgc aacaaactat ttgtcgcagg aataaagatg 180

gctgctgaac cagtagaaga caattgcatc aactttgtgg caatgaaatt tattgacaat 240

acgctttact ttatagctga agatgatgaa aacctggaat cagattactt tggcaagctt 300

gaatctaaat tatcagtcat aagaaatttg aatgaccaag ttctcttcat tgaccaagga 360

aatcggcctc tatttgaaga tatgactgat tctgactgta gagataatgc accccggacc 420

atatttatta taagtatgta taaagatagc cagcctagag gtatggctgt aactatctct 480

gtgaagtgtg agaaaatttc aactctctcc tgtgagaaca aaattatttc ctttaaggaa 540

atgaatcctc ctgataacat caaggataca aaaagtgaca tcatattctt tcagagaagt 600

gtcccaggac atgataataa gatgcaattt gaatcttcat catacgaagg atactttcta 660

gcttgtgaaa aagagagaga cctttttaaa ctcattttga aaaaagagga tgaattgggg 720

gatagatcta taatgttcac tgttcaaaac gaagactagc tattaaaatt tcatgccggg 780

cgcagtggct cacgcctgta atcccagccc tttgggaggc tgaggcgggc agatcaccag 840

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gcaggagaat cacttgcact ccggaggtag aggttgtggt gagccgagat tgcaccattg 1020

cgctctagcc tgggcaacaa cagcaaaact ccatctcaaa aaataaaata aataaataaa 1080

caaataaaaa attcataatg tg 1102

<210> 401
<211> 1437
<212> DNA
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ggcgaaaatg ggaaccatat tattttcggt ggaccttgga aataggggct agagagagca 900

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<211> 630
<212> DNA
<213> Homo sapiens

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atttaacaat gtcaggctgc tgttccaaaa 630

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<211> 485
<212> DNA
<213> Homo sapiens

<400> 467
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<211> 1748
<212> DNA
<213> Homo sapiens

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acgggcttcg ggagggccct ggggggacag ttccgagtca twwacctctt cactgcggtc 180

accctgagyg tcaccaccgt cctgaccctg gtcagcatcc ctgagaggcc gctgcggccg 240

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<213> Homo sapiens

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<223> n is a, c, g, t or u

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caaagcgatg agtctccagc aaaagccggg ggaacttttt cgcggcgctc gggatcctga 180

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g 241

<210> 471
<211> 389

<212> DNA
<213> Homo sapiens

<400> 471
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ttcatttttt atccttttc tgttatattt tatctaacaa ccttgatcca tgacaatgtg 180

aaaaaaaaag acaataagtt ttcttctatg tgacttacag caacatagca agtatgttac 240

gatattaaat attttatttt ctaacctttc aaaattaaga acttatgaat aaatgagatg 300

actctcagaa tatgaacaga aaagtctact tctgaacata aaaatgtaat cagaaacaat 360

gtttccacag aataagatgt aaaggtatc 389

<210> 472
<211> 491
<212> DNA
<213> Homo sapiens

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<221> misc_feature
<222> (487)..(487)
<223> n is a, c, g, t or u

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<210> 473
<211> 557
<212> DNA
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<220>
<221> misc_feature
<222> (499)..(499)
<223> n is a, c, g, t or u

<220>
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ctttctcatt cggngag 557

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tcacacaatt acactacaat ctgataggag tggtaaaacc agccaatgga atccaggtaa 180

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